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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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R C van Dijk

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Anmelder/Applicant(s)/Demandeur(s):

DeveloGen Aktiengesellschaft für
entwicklungsbiologische Forschung
Rudolf-Wissell-Strasse 28
37079 Göttingen
ALLEMAGNE

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Fwd, Adk3, Gdh, or OSBP homologous proteins involved in the regulation of energy homeostasis

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WEICKMANN & WEICKMANN

Patentanwälte
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DIPL.-ING. H. WEICKMANN (bis 31.1.01)
DIPL.-ING. F. A. WEICKMANN
DIPL.-CHEM. B. HUBER
DR.-ING. H. LISKA
DIPL.-PHYS. DR. J. PRECHTEL
DIPL.-CHEM. DR. B. BÖHM
DIPL.-CHEM. DR. W. WEISS
DIPL.-PHYS. DR. J. TIESMEYER
DIPL.-PHYS. DR. M. HERZOG
DIPL.-PHYS. B. RUTTENSPERGER
DIPL.-PHYS. DR.-ING. V. JORDAN
DIPL.-CHEM. DR. M. DEY
DIPL.-FORSTW. DR. J. LACHNIT

Unser Zeichen:
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Anmelder:
DeveloGen Aktiengesellschaft für
entwicklungsbiologische Forschung
Rudolf-Wissell-Straße 28

37079 Göttingen
DEUTSCHLAND

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Postfach 860 820, 81635 München, Deutschland, Tel. (089) 45563-0, Fax (089) 45563-999, ...

Fwd, Adk3, Gdh, or OSBP homologous proteins involved in the regulation of energy homeostasis

Description

This invention relates to the use of nucleic acid sequences encoding fwd, Adk3, Gdh, or OSBP homologous proteins, and the polypeptides encoded thereby and to the use thereof and to the use of effectors thereof in the diagnosis, study, prevention, and treatment of metabolic diseases or dysfunctions, for example, but not limited to, such as metabolic syndrome including obesity, diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones.

There are several metabolic diseases of human and animal metabolism, e.g., obesity and severe weight loss, that relate to energy imbalance where caloric intake versus energy expenditure is imbalanced. Obesity is one of the most prevalent metabolic disorders in the world. It is still a poorly understood human disease that becomes as a major health problem more and more relevant for western society. Obesity is defined as a body weight more than 20% in excess of the ideal body weight, frequently resulting in a significant impairment of health. Obesity may be measured by body mass index, an indicator of adiposity or fatness. Further parameters for defining obesity are waist circumferences, skinfold thickness and bioimpedance (see, inter alia, Kopelman (1999), loc. cit.). It is associated with an increased risk for cardiovascular disease, hypertension, diabetes mellitus Type II, hyperlipidaemia and an increased mortality rate. Besides severe risks of illness, individuals suffering from obesity are often isolated socially.

Obesity is influenced by genetic, metabolic, biochemical, psychological, and behavioral factors and can be caused by different reasons such as

non-insulin dependent diabetes, increase in triglycerides, increase in carbohydrate bound energy and low energy expenditure. As such, it is a complex disorder that must be addressed on several fronts to achieve lasting positive clinical outcome. Since obesity is not to be considered as a single disorder but as a heterogeneous group of conditions with (potential) multiple causes, it is also characterized by elevated fasting plasma insulin and an exaggerated insulin response to oral glucose intake (Koltermann J., (1980) Clin. Invest 65, 1272-1284). A clear involvement of obesity in type 2 diabetes mellitus can be confirmed (Kopelman P.G., (2000) Nature 404, 635-643).

Triglycerides and glycogen are used as the body's fuel energy storage. Glycogen is a large branched polymer of glucose residues that is mainly stored in liver and muscle cells. Glycogen synthesis and degradation is central to the control of the blood glucose level.

Triglycerides are stored in the cytoplasm of adipocytes. Adipocytes are specialized for the synthesis, storage and mobilization of triglycerides. The glycogen and triglyceride metabolism is highly regulated and their interplay is essential for the energy homeostasis of the body. A high glucose level in the adipose cell results in the synthesis of triglycerides as fuel store. A low intracellular glucose level leads to a release of fatty acids, which can be used as substrates for the beta-oxidation to generate energy. Glycogen levels in cells are more variable than triglyceride levels because the turnover of glycogen is higher. Triglycerides are used as long term energy donors once the glycogen stores run low.

Insulin amongst other hormones plays a key role in the regulation of the fuel metabolism. High blood glucose levels stimulate the secretion of insulin by pancreatic beta-cells. Insulin leads to the storage of glycogen and triglycerides and to the synthesis of proteins. The entry of glucose into muscles and adipose cells is stimulated by insulin.

In patients who suffer from diabetes mellitus either the amount of insulin produced by the pancreatic islet cells is to low (Diabetes Type 1 or insulin dependent diabetes mellitus IDDM) or liver and muscle cells loose their ability to respond to normal blood insulin levels (insulin resistance). In the next stage pancreatic cells become unable to produce sufficient amounts of insulin (Diabetes Type II or non insulin dependent diabetes mellitus NIDDM).

Hyperlipidemia and elevation of free fatty acids correlate clearly with the 'Metabolic Syndrome', which is defined as the linkage between several diseases, including obesity an insulin resistance. This often occurs in the same patients and is a major risk factor for development of Type 2 diabetes and cardiovascular disease. It was suggested that the control of lipid levels and glucose levels is required to treat Type 2 Diabetes, heart disease, and other occurances of Metabolic Syndrome (see, for example, Santomauro A. T. et al., (1999) Diabetes, 48(9):1836-1841).

The molecular factors regulating food intake and body weight balance are incompletely understood. Even if several candidate genes have been described which are supposed to influence the homeostatic system(s) that regulate body mass/weight, like leptin, VCPI, VCPL or the peroxisome proliferator-activated receptor-gamma co-activator, the distinct molecular mechanisms and/or molecules influencing obesity or body weight/body mass regulations are not known. In addition, several single-gene mutations resulting in obesity have been described in mice, implicating genetic factors in the etiology of obesity (Friedman J. M. and Leibel R. L., (1992) Cell 69(2): 217-220). In the ob mouse a single gene mutation (obese) results in profound obesity, which is accompanied by diabetes (Friedman J. M. et. al., (1991) Genomics 11: 1054-1062).

Therefore, the technical problem underlying the present invention was to provide for means and methods for modulating (pathological) metabolic

conditions influencing body-weight regulation and/or energy homeostatic circuits. The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to genes with novel functions in body-weight regulation, energy homeostasis, metabolism, and obesity. The present invention discloses specific genes involved in the regulation of body-weight, energy homeostasis, metabolism, and obesity, as well as related diseases such as diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones. In particular, the present invention describes the human fwd, Adk3, Gdh, or OSBP homologous genes as being involved in those conditions mentioned above.

The synthesis of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], the immediate precursor of intracellular signals generated by calcium-mobilizing hormones and growth factors, is initiated by the conversion of phosphatidylinositol to phosphatidylinositol 4-phosphate [PtdIns(4)P] by phosphatidylinositol 4-kinase (PtdIns 4-kinase) (Meyers R. and Cantley L. C., (1997) J. Biol. Chem. 272, 4384-4390). Targeting of Golgi-specific pleckstrin homology domains of oxysterol binding protein (OSBP) involves both PtdIns 4-kinase-dependent and -independent components (Levine T. P. and Munro S., (2002) Curr Biol 12(9):695-704).

Biochemical analyses indicated that Phosphatidyl inositol 4-kinase beta (PI4Kbeta, PIK4CB) is a type III enzyme that is sensitive to wortmannin (Meyers R. and Cantley L. C., supra). PI4Kbeta is localized in the cytosol and also present in the Golgi region (Wong K. et al., (1999) J Biol Chem 1997 May 16;272(20):13236-13241). PI4Kbeta is ubiquitously expressed, with highest expression in heart, pancreas, and skeletal muscle.

PI4Kbeta (PIK4CB, PI4K type III) was primarily found in the Golgi, but it was also present in the walls of numerous large perinuclear vesicles. Co-expression of a catalytically inactive PI4Kbeta inhibited the development of this vesicular phenotype. PI4Kbeta is involved in vesicular trafficking (Zhao X. et al., (2001) J Biol Chem 276(43):40183-40189).

Adenylate kinases regulate the adenine and guanine nucleotide compositions within a cell by catalyzing the reversible transfer of phosphate group among these nucleotides. Three isozymes of adenylate kinase have been identified in vertebrates, adenylate isozyme 1 (AK1), 2 (AK2) and 3 (AK3). Expression of these isozymes is tissue-specific and developmentally regulated.

AK3 is present in the mitochondrial matrix and prefers GTP over ATP as the substrate (Wilson D. E. et al. (1976) Ann Hum Genet 39(3):305-313). In the failing myocardium, phosphotransfer activities of creatine kinase, adenylate kinase, 3-phosphoglycerate kinase and pyruvate kinase, which collectively deliver ATP and remove ADP from myofibrillar ATPases, were depressed, when compared to normal controls. As these enzymatic systems are collectively required for adequate delivery of high-energy phosphoryl to, and removal of end-products from, cellular ATPases, the cumulative deficit in their flux capacities may provide a bioenergetic basis for impaired contraction-relaxation in the failing heart (Dzeja P. P. et al., (1999) Mol Cell Biochem 201(1-2):33-40).

L-glutamate dehydrogenase (GLUD) has a central role in nitrogen metabolism in plants and animals. Glutamate dehydrogenase is found in all organisms and catalyzes the oxidative deamination of 1-glutamate to 2-oxoglutarate (Smith T. J. et al., (2001) J Mol Biol 307(2):707-720). Glutamate, the main substrate of GLUD, is present in brain in concentrations higher than in other organs. In nervous tissue, GLUD

appears to function in both the synthesis and the catabolism of glutamate and perhaps in ammonia detoxification (Mavrothalassitis G. et al., (1988) Proc Natl Acad Sci U S A 85(10):3494-3498).

Polyamines are of importance to control glutamate dehydrogenase activity under physiological conditions. Spermidine is a potent inhibitor of glutamate synthesis, resulting in about 80% decrease of enzyme activity (Jarzyna R. et al., (1994) Biochem Pharmacol 47(8):1387-1393). It is suggested that naturally occurring polyamines acts as physiological modulator of GDH activity in pancreatic beta cells (Bryla J. et al., (1994) Metabolism 43(9):1187-1195). Enzymes of nitrogen metabolism showed reduced activities in brown adipose tissue (BAT) of obese rats, including glutamate dehydrogenase (Serra F. et al., (1994) Biochem Mol Biol Int 32(6):1173-1178). Glutamate dehydrogenase is important in normal glucose homeostasis. Mutations of GDH (gain-of-function) result in hyperinsulinism / hyperammonemia syndrome. Glutamate, generated by this enzyme, participates in insulin secretion as a glucose-derived metabolic messenger (Maechler P. and Wollheim C. B., (2000) J Physiol 529 Pt 1:49-56). Constitutively activated GDH enhances oxidation of glutamate, which is intracellularly converted from glutamine to alpha-ketoglutarate, a tricarboxylic acid cycle substrate, which thereby stimulates insulin secretion (Tanizawa Y. et al., (2002) Diabetes 51(3):712-717).

The OSBPL1A and OSBP2 genes encode members of the oxysterol-binding protein (OSBP) family, a group of intracellular lipid receptors. Most members contain an N-terminal pleckstrin homology domain and a highly conserved C-terminal OSBP-like sterol-binding domain, although some members contain only the sterol-binding domain (e.g. OSBP2). The OSBP2 protein contains only the sterol-binding domain. In vitro studies have shown that the encoded protein can bind strongly to phosphatic acid and weakly to phosphatidylinositol 3-phosphate, but cannot bind to 25-hydroxycholesterol. The protein associates with the Golgi apparatus.

ORP2, an oxysterol binding protein related protein, is a regulator of cellular sterol homeostasis and intracellular membrane trafficking (Laitinen S. et al. (2002) J Lipid Res 43(2):245-255).

So far, it has not been described that the proteins of the invention and homologous proteins are involved in the regulation of energy homeostasis and body-weight regulation and related disorders, and thus, no functions in metabolic diseases and dysfunctions and other diseases as listed above have been discussed.

In this invention we refer to the proteins encoded by Drosophila fwd, Adk3, Gdh, or OSBP genes and homologous orthologs, preferably human and murine homologous polypeptides or proteins or sequences encoding those proteins as proteins of the invention.

The present invention discloses that fwd, Adk3, Gdh, or OSBP homologous proteins are regulating the energy homeostasis and fat metabolism especially the metabolism and storage of triglycerides and glycogen, and polynucleotides, which identify and encode the proteins disclosed in this invention. The invention also relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides of the invention. The invention also relates to the use of these polynucleotides, polypeptides and effectors thereof in the diagnosis, study, prevention, and treatment of metabolic diseases or dysfunctions, for example, but not limited to, metabolic syndrome including obesity, diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones.

Fwd, Adk3, Gdh, or OSBP homologous proteins and nucleic acid molecules coding therefore are obtainable from insect or vertebrate species, e.g. mammals or birds. Particularly preferred are nucleic acids encoding the human fwd, Adk3, Gdh, or OSBP homologs (in particular the human

isoforms of phosphatidylinositol 4-kinase, catalytic, beta polypeptide, of adenylate kinase 3, of adenylate kinase 3 alpha like, of glutamate dehydrogenase 1, of glutamate dehydrogenase 2, of oxysterol binding protein-like 1A, and of oxysterol binding protein-like 2).

The invention particularly relates to a nucleic acid molecule encoding a polypeptide contributing to regulating the energy homeostasis and the metabolism of triglycerides and glycogen, wherein said nucleic acid molecule comprises

(a) the nucleotide sequence of Drosophila fwd, Adk3, Gdh, or OSBP, human fwd, Adk3, Gdh, or OSBP homologs (in particular the human isoforms of phosphatidylinositol 4-kinase, catalytic, beta polypeptide, of adenylate kinase 3, of adenylate kinase 3 alpha like, of glutamate dehydrogenase 1, of glutamate dehydrogenase 2, of oxysterol binding protein-like 1A, and of oxysterol binding protein-like 2), and/or a sequence complementary thereto,

(b) a nucleotide sequence which hybridizes at 50°C in a solution containing 1 x SSC and 0.1% SDS to a sequence of (a),

(c) a sequence corresponding to the sequences of (a) or (b) within the degeneration of the genetic code,

(d) a sequence which encodes a polypeptide which is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably at least 98% and up to 99,6% identical to the amino acid sequences of the fwd, Adk3, Gdh, or OSBP protein, preferably of the human fwd, Adk3, Gdh, or OSBP homologs (in particular the human isoforms of phosphatidylinositol 4-kinase, catalytic, beta polypeptide, of adenylate kinase 3, of adenylate kinase 3 alpha like, of glutamate dehydrogenase 1, of glutamate dehydrogenase 2, of oxysterol binding protein-like 1A, and of oxysterol binding protein-like 2),

(e) a sequence which differs from the nucleic acid molecule of (a) to (d) by mutation and wherein said mutation causes an alteration, deletion, duplication and/or premature stop in the encoded polypeptide or

(f) a partial sequence of any of the nucleotide sequences of (a) to (e) having a length of at least 15 bases, preferably at least 20 bases, more preferably at least 25 bases and most preferably at least 50 bases.

The present invention relates to genes with novel functions in body-weight regulation, energy homeostasis, metabolism, and obesity, fragments of said genes, polypeptides encoded by said genes or fragments thereof, and effectors e.g. antibodies, biologically active nucleic acids, such as antisense molecules, RNAi molecules or ribozymes, aptamers, peptides or low-molecular weight organic compounds recognizing said polynucleotides or polypeptides.

The ability to manipulate and screen the genomes of model organisms such as the fly Drosophila melanogaster provides a powerful tool to analyze biological and biochemical processes that have direct relevance to more complex vertebrate organisms due to significant evolutionary conservation of genes, cellular processes, and pathways (see, for example, Adams M. D. et al., (2000) Science 287: 2185-2195). Identification of novel gene functions in model organisms can directly contribute to the elucidation of correlative pathways in mammals (humans) and of methods of modulating them. A correlation between a pathology model (such as changes in triglyceride levels as indication for metabolic syndrome including obesity) and the modified expression of a fly gene can identify the association of the human ortholog with the particular human disease.

In one embodiment, a forward genetic screen is performed in fly displaying a mutant phenotype due to misexpression of a known gene (see, Johnston

Nat Rev Genet 3: 176-188 (2002); Rorth P., (1996) Proc Natl Acad Sci U S A 93: 12418-12422). In this invention, we have used a genetic screen to identify mutations of fwd, Adk3, Gdh, or OSBP homologous genes that cause changes in the body weight which is reflected by a significant change of triglyceride levels. Additionally glycogen levels are analysed.

One resource for screening was a Drosophila melanogaster stock collection of EP-lines. The P-vector of this collection has Gal4-UAS-binding sites fused to a basal promoter that can transcribe adjacent genomic Drosophila sequences upon binding of Gal4 to UAS-sites (Brand & Perrimon (1993) Development 118:401-415; Rorth P., (1996) Proc Natl Acad Sci U S A 93:12418-12422). This enables the EP-line collection for overexpression of endogenous flanking gene sequences. In addition, without activation of the UAS-sites, integration of the EP-element into the gene is likely to cause a reduction of gene activity, and allows determining its function by evaluating the loss-of-function phenotype.

To isolate genes with a function in energy homeostasis, several thousand EP-lines were tested for their triglyceride/glycogen content after a prolonged feeding period (see Examples for more detail). Lines with significantly changed triglyceride/glycogen content were selected as positive candidates for further analysis. The change of triglyceride/glycogen content due to the loss of a gene function suggests gene activities in energy homeostasis in a dose dependent manner that control the amount of energy stored as triglycerides or glycogens.

In this invention, the content of triglycerides and glycogen of a pool of flies with the same genotype was analyzed after feeding for six days using a triglyceride and a glycogen assay. Male flies homozygous for the integration of vectors for Drosophila lines HD-EP(3)30148, HD-EP(3)35207, and HD-EP(2)25831, and heterozygous for the integration of vectors for Drosophila lines HD-EP(3)36627, were analyzed in assays

measuring the triglyceride and glycogen contents of these flies, illustrated in more detail in the EXAMPLES section. The results of the glycogen content analysis are shown in FIGURES 1, 4, 7, and 10.

Genomic DNA sequences were isolated that are localized to the EP vector (herein HD-EP(3)30148, HD-EP(3)35207, HD-EP(2)25831, and HD-EP(3)36627) integration. Using those isolated genomic sequences public databases like Berkeley Drosophila Genome Project (GadFly; see also FlyBase (1999) Nucleic Acids Research 27:85-88) were screened thereby identifying the integration site of the vectors, and the corresponding gene, described in more detail in the EXAMPLES section. The molecular organization of the gene is shown in FIGURES 2, 5, 8, and 11.

The invention also encompasses polynucleotides that encode the proteins of the invention and homologous proteins. Accordingly, any nucleic acid sequence, which encodes the amino acid sequences of the proteins of the invention and homologous proteins, can be used to generate recombinant molecules that express the proteins of the invention and homologous proteins. In a particular embodiment, the invention encompasses a nucleic acid encoding Drosophila fwd, Adk3, Gdh, or OSBP or human fwd, Adk3, Gdh, or OSBP homologs; referred to herein as the proteins of the invention. It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding the proteins, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. The invention contemplates each and every possible variation of nucleotide sequence that can be made by selecting combinations based on possible codon choices.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those of the polynucleotide encoding the proteins of the

invention, under various conditions of stringency. Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe, as taught in Wahl & Berger (1987: Methods Enzymol. 152:399-407) and Kimmel (1987; Methods Enzymol. 152:507-511), and may be used at a defined stringency. Preferably, hybridization under stringent conditions means that after washing for 1 h with 1 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 65°C, particularly for 1 h in 0.2 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 65°C, a positive hybridization signal is observed. Altered nucleic acid sequences encoding the proteins which are encompassed by the invention include deletions, insertions or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent protein.

The encoded proteins may also contain deletions, insertions or substitutions of amino acid residues, which produce a silent change and result in functionally equivalent proteins. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of the protein is retained. Furthermore, the invention relates to peptide fragments of the proteins or derivatives thereof such as cyclic peptides, retro-inverso peptides or peptide mimetics having a length of at least 4, preferably at least 6 and up to 50 amino acids.

Also included within the scope of the present invention are alleles of the genes encoding the proteins of the invention and homologous proteins. As used herein, an 'allele' or 'allelic sequence' is an alternative form of the gene, which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structures or function may or may not be altered. Any given gene may

have none, one or many allelic forms. Common mutational changes, which give rise to alleles, are generally ascribed to natural deletions, additions or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence.

The nucleic acid sequences encoding the proteins of the invention and homologous proteins may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements.

In order to express a biologically active protein, the nucleotide sequences encoding the proteins or functional equivalents, may be inserted into appropriate expression vectors, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods, which are well known to those skilled in the art, may be used to construct expression vectors containing sequences encoding the proteins and the appropriate transcriptional and translational control elements. Regulatory elements include for example a promoter, an initiation codon, a stop codon, a mRNA stability regulatory element, and a polyadenylation signal. Expression of a polynucleotide can be assured by (i) constitutive promoters such as the Cytomegalovirus (CMV) promoter/enhancer region, (ii) tissue specific promoters such as the insulin promoter (see, Soria et al., 2000, Diabetes 49:157), SOX2 gene promotor (see Li et al., (1998) Curr. Biol. 8:971-4), Msi-1 promotor (see Sakakibara et al., (1997) J. Neuroscience 17:8300-8312), alpha-cardia myosin heavy chain promotor or human atrial natriuretic factor promotor (Klug et al., (1996) J. clin. Invest 98:216-24; Wu et al., (1989) J. Biol. Chem. 264:6472-79) or (iii) inducible promoters such as the tetracycline inducible system. Expression vectors can also contain a selection agent or marker gene that confers antibiotic resistance such as the neomycin, hygromycin or puromycin resistance genes. These methods include in vitro recombinant

DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y. and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

In a further embodiment of the invention, natural, modified or recombinant nucleic acid sequences encoding the proteins of the invention and homologous proteins may be ligated to a heterologous sequence to encode a fusion protein.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding the proteins or fusion proteins. These include, but are not limited to, micro-organisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus, adenovirus, adeno-associated virus, lentiverus, retrovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or PBR322 plasmids); or animal cell systems.

The presence of polynucleotide sequences of the invention in a sample can be detected by DNA-DNA or DNA-RNA hybridization and/or amplification using probes or portions or fragments of said polynucleotides. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences specific for the gene to detect transformants containing DNA or RNA encoding the corresponding protein. As used herein 'oligonucleotides' or 'oligomers' refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting polynucleotide sequences include oligo-labeling, nick translation, end-labeling of labeled RNA probes, PCR amplification using a labeled nucleotide, or enzymatic synthesis. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, Mich.); Promega (Madison Wis.); and U.S. Biochemical Corp., (Cleveland, Ohio).

The presence of proteins of the invention in a sample can be determined by immunological methods or activity measurement. A variety of protocols for detecting and measuring the expression of proteins, using either polyclonal or monoclonal antibodies specific for the protein or reagents for determining protein activity are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the protein is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, Minn.) and Maddox, D. E. et al. (1983; J. Exp. Med. 158:1211-1216).

Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent or chromogenic agents as well as substrates, co-factors, inhibitors, magnetic particles, and the like.

The nucleic acids encoding the proteins of the invention can be used to generate transgenic animal or site specific gene modifications in cell lines. Transgenic animals may be made through homologous recombination,

where the normal locus of the genes encoding the proteins of the invention is altered. Alternatively, a nucleic acid construct is randomly integrated into the genome. Vectors for stable integration include plasmids, retrovirusses and other animal virusses, YACs, and the like. The modified cells or animal are useful in the study of the function and regulation of the proteins of the invention. For example, a series of small deletions and/or substitutions may be made in the genes that encode the proteins of the invention to determine the role of particular domains of the protein, functions in pancreatic differentiation, etc.

Specific constructs of interest include anti-sense molecules, which will block the expression of the proteins of the invention, or expression of dominant negative mutations. A detectable marker, such as for example lac-Z, may be introduced in the locus of the genes of the invention, where upregulation of expression of the genes of the invention will result in an easily detected change in phenotype.

One may also provide for expression of the genes of the invention or variants thereof in cells or tissues where it is not normally expressed or at abnormal times of development. In addition, by providing expression of the proteins of the invention in cells in which they are not normally produced, one can induce changes in cell behavior.

DNA constructs for homologous recombination will comprise at least portions of the genes of the invention with the desired genetic modification, and will include regions of homology to the target locus. DNA constructs for random integration need not include regions of homology to mediate recombination. Conveniently, markers for positive and/or negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For embryonic stem (ES) cells, an ES cell line may be employed, or embryonic cells may be obtained freshly from a host, e.g. mouse, rat, guinea pig etc.

Such cells are grown on an appropriate fibroblast-feeder layer or grown in presence of leukemia inhibiting factor (LIF).

When ES or embryonic cells or somatic pluripotent stem cells have been transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination or integration of the construct. Those colonies that are positive may then be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting offspring screened for the construct. By providing for a different phenotype of the blastocyst and the genetically modified cells, chimeric progeny can be readily detected. The chimeric animals are screened for the presence of the modified gene and males and females having the modification are mated to produce homozygous progeny. If the gene alterations cause lethality at some point in development, tissues or organs can be maintained as allogenic or congenic grafts or transplants, or in vitro culture. The transgenic animals may be any non-human mammal, such as laboratory animal, domestic animals, etc. The transgenic animals may be used in functional studies, drug screening, etc.

Diagnostics and Therapeutics

The data disclosed in this invention show that the nucleic acids and proteins of the invention and effector molecules thereof are useful in diagnostic and therapeutic applications implicated, for example, but not

limited to, metabolic syndrome including obesity, diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones.. Hence, diagnostic and therapeutic uses for the proteins of the invention nucleic acids and proteins of the invention are, for example but not limited to, the following: (i) protein therapeutic, (ii) small molecule drug target, (iii) antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) diagnostic and/or prognostic marker, (v) gene therapy (gene delivery/gene ablation), (vi) research tools, and (vii) tissue regeneration in vitro and in vivo (regeneration for all these tissues and cell types composing these tissues and cell types derived from these tissues).

The nucleic acids and proteins of the invention and effectors thereof are useful in diagnostic and therapeutic applications implicated in various applications as described below. For example, but not limited to, cDNAs encoding the proteins of the invention and particularly their human homologues may be useful in gene therapy, and the proteins of the invention and particularly their human homologues may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from, for example, but not limited to, in metabolic disorders as described above.

The nucleic acids of the invention or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acids or the proteins are to be assessed. Further antibodies that bind immunospecifically to the novel substances of the invention may be used in therapeutic or diagnostic methods.

For example, in one aspect, antibodies, which are specific for the proteins of the invention and homologous proteins, may be used directly as an effector, e.g. an antagonist or indirectly as a targeting or delivery

mechanism for bringing a pharmaceutical agent to cells or tissue which express the protein. The antibodies may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric single chain, Fab fragments, and fragments produced by a Fab expression library. Neutralising antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with the protein or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. It is preferred that the peptides, fragments or oligopeptides used to induce antibodies to the protein have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids.

Monoclonal antibodies to the proteins may be prepared using any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Köhler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R. J. et al. Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S. P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of 'chimeric antibodies', the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S. L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M. S. et al (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature 314:452-454). Alternatively, techniques

described for the production of single chain antibodies may be adapted, using methods known in the art, to produce single chain antibodies specific for the proteins of the invention and homologous proteins. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, D. R. (1991) Proc. Natl. Acad. Sci. 88:11120-3). Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

Antibody fragments which contain specific binding sites for the proteins may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by Pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse, W. D. et al. (1989) Science 254:1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding and immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between the protein and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reacive to two non-interfering protein epitopes are preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides or fragments thereof or nucleic acid effector molecules such as antisense molecules, aptamers, RNAi molecules or ribozymes may be used for therapeutic purposes. In one aspect, aptamers, i.e. nucleic acid molecules, which are capable of binding to a protein of the invention and modulating its activity, may be generated by a screening and selection procedure involving the use of combinatorial nucleic acid libraries.

In a further aspect, antisense molecules may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding the proteins of the invention and homologous proteins. Thus, antisense molecules may be used to modulate protein activity or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding the proteins. Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods, which are well known to those skilled in the art, can be used to construct recombinant vectors, which will express antisense molecules complementary to the polynucleotides of the genes encoding the proteins of the invention and homologous proteins. These techniques are described both in Sambrook et al. (supra) and in Ausubel et al. (supra). Genes encoding the proteins of the invention and homologous proteins can be turned off by transforming a cell or tissue with expression vectors, which express high levels of polynucleotides that encode the proteins of the invention and homologous proteins or fragments thereof. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by

endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, e.g. DNA, RNA or nucleic acid analogues such as PNA, to the control regions of the genes encoding the proteins of the invention and homologous proteins, i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it cause inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J. E. et al. (1994) In; Huber, B. E. and B. I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, N.Y.). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples, which may be used, include engineered hammerhead motif ribozyme molecules that can be specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding the proteins of the invention and homologous proteins. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing

the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Nucleic acid effector molecules, e.g. antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences. Such DNA sequences may be incorporated into a variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues. RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5′ and/or 3′ ends of the molecule or modifications in the nucleobase, sugar and/or phosphate moieties, e.g. the use of phosphorothioate or 2′ O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using

methods, which are well known in the art. Any of the therapeutic methods described above may be applied to any suitable subject including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of the nucleic acids and the proteins of the invention and homologous nucleic acids or proteins, antibodies to the proteins of the invention and homologous proteins, mimetics, agonists, antagonists or inhibitors of the proteins of the invention and homologous proteins or nucleic acids. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone or in combination with other agents, drugs or hormones. The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations, which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compounds, the therapeutically effective does can be estimated initially either in cell culture assays, e.g., of preadipocyte cell lines or in animal models, usually mice, rabbits, dogs or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically effective dose refers to that amount of active ingredient, for example the nucleic acids or the proteins of the invention or fragments thereof or antibodies, which is sufficient for treating a specific condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions, which exhibit large therapeutic indices, are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage from employed, sensitivity of the patient, and the route of administration. The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors, which may be taken into account, include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy.

Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week or once every two weeks depending on half-life and clearance rate of the particular formulation. Normal dosage amounts may vary from 0.1 to 100,000 microg, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

In another embodiment, antibodies which specifically bind to the proteins may be used for the diagnosis of conditions or diseases characterized by or associated with over- or underexpression of the proteins of the invention and homologous proteins or in assays to monitor patients being treated with the proteins of the invention and homologous proteins, or effectors thereof, e.g. agonists, antagonists, or inhibitors. Diagnostic assays include methods which utilize the antibody and a label to detect the protein in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring proteins are known in the art and provide a basis for diagnosing altered or abnormal levels of gene expression. Normal or standard values for gene expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibodies to the protein under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric means. Quantities of protein expressed in control

and disease, samples e.g. from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides specific for the proteins of the invention and homologous proteins may be used for diagnostic purposes. The polynucleotides, which may be used, include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which gene expression may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess gene expression, and to monitor regulation of protein levels during therapeutic intervention.

In one aspect, hybridization with probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding the proteins of the invention and homologous proteins or closely related molecules, may be used to identify nucleic acid sequences which encode the respective protein. The hybridization probes of the subject invention may be DNA or RNA and are preferably derived from the nucleotide sequence of the polynucleotide encoding the proteins of the invention or from a genomic sequence including promoter, enhancer elements, and introns of the naturally occurring gene. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as ^{32}P or ^{35}S or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences specific for the proteins of the invention and homologous nucleic acids may be used for the diagnosis of conditions or diseases, which are associated with the expression of the proteins. Examples of such conditions or diseases include, but are not limited to, pancreatic diseases and disorders, including diabetes. Polynucleotide

sequences specific for the proteins of the invention and homologous proteins may also be used to monitor the progress of patients receiving treatment for pancreatic diseases and disorders, including diabetes. The polynucleotide sequences may be used qualitative or quantitative assays, e.g. in Southern or Northern analysis, dot blot or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect altered gene expression.

In a particular aspect, the nucleotide sequences specific for the proteins of the invention and homologous nucleic acids may be useful in assays that detect activation or induction of various metabolic diseases or dysfunctions, for example, obesity, diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones. The nucleotide sequences may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. The presence of altered levels of nucleotide sequences encoding the proteins of the invention and homologous proteins in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disease associated with expression of the proteins of the invention and homologous proteins, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence or a fragment thereof, which is specific for the nucleic acids encoding the proteins of the invention and homologous nucleic acids, under conditions suitable for

hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease. Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that, which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to metabolic diseases such as described above the presence of an unusual amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the metabolic diseases and disorders.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding the proteins of the invention and homologous proteins may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5prime.fwdarw.3prime) and another with antisense (3prime.rarw.5prime), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers or even a degenerate pool of oligomers may be

employed under less stringent conditions for detection and/or quantification of closely related DNA or RNA sequences.

In another embodiment of the invention, the nucleic acid sequences may also be used to generate hybridization probes, which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. Such techniques include FISH, FACS or artificial chromosome constructions, such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C. M. (1993) Blood Rev. 7:127-134, and Trask, B. J. (1991) Trends Genet. 7:149-154. FISH (as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, N.Y.). The results may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding the proteins of the invention on a physical chromosomal map and a specific disease or predisposition to a specific disease, may help to delimit the region of DNA associated with that genetic disease.

The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals. An analysis of polymorphisms, e.g. single nucleotide polymorphisms may be carried out. Further, in situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms or parts thereof, by physical mapping. This provides

valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R. A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequences of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier or affected individuals.

In another embodiment of the invention, the proteins of the invention, their catalytic or immunogenic fragments or oligopeptides thereof, an in vitro model, a genetically altered cell or animal, can be used for screening libraries of compounds in any of a variety of drug screening techniques. One can identify effectors, e.g. receptors, enzymes, proteins, ligands, or substrates that bind to, modulate or mimic the action of one or more of the proteins of the invention. The protein or fragment thereof employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellulary. The formation of binding complexes, between the proteins of the invention and the agent tested, may be measured. Agents could also, either directly or indirectly, influence the activity of the proteins of the invention.

In vivo, the enzymatic kinase activity of the unmodified polypeptides of PIK4CB and AK3 towards a substrate can be enhanced by appropriate stimuli, triggering the phosphorylation of PIK4CB and AK3. This may be induced in the natural context by extracellular or intracellular stimuli, such as signaling molecules or environmental influences. One may generate a system containing activated PIK4CB or AK3, may it be an organism, a tissue, a culture of cells or cell-free environment, by exogenously applying this stimulus or by mimicking this stimulus by a variety of the techniques, some of them described further below. A system containing activated

PIK4CB or AK3 may be produced (i) for the purpose of diagnosis, study, prevention, and treatment of diseases and disorders related to body-weight regulation and thermogenesis, for example, but not limited to, metabolic diseases such as obesity, as well as related disorders such as eating disorder, cachexia, diabetes mellitus, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, and sleep apnea.

In addition activity of fwd, Adk3, Gdh, or OSBP against its physiological substrate(s) or derivatives thereof could be measured in cell-based assays. Agents may also interfere with posttranslational modifications of the protein, such as phosphorylation and dephosphorylation, farnesylation, palmitoylation, acetylation, alkylation, ubiquitination, proteolytic processing, subcellular localization and degradation. Moreover, agents could influence the dimerization or oligomerization of the proteins of the invention or, in a heterologous manner, of the proteins of the invention with other proteins, for example, but not exclusively, docking proteins, enzymes, receptors, or translation factors. Agents could also act on the physical interaction of the proteins of this invention with other proteins, which are required for protein function, for example, but not exclusively, their downstream signaling.

Methods for determining protein-protein Interaction are well known in the art. For example binding of a fluorescently labeled peptide derived from the interacting protein to the protein of the Invention, or vice versa, could be detected by a change in polarisation. In case that both binding partners, which can be either the full length proteins as well as one binding partner as the full length protein and the other just represented as a peptide are fluorescently labeled, binding could be detected by fluorescence energy transfer (FRET) from one fluorophore to the other. In addition, a variety of commercially available assay principles suitable for detection of protein-protein Interaction are well known In the art, for example but not

exclusively AlphaScreen (PerkinElmer) or Scintillation Proximity Assays (SPA) by Amersham. Alternatively, the interaction of the proteins of the invention with cellular proteins could be the basis for a cell-based screening assay, in which both proteins are fluorescently labeled and interaction of both proteins is detected by analysing cotranslocation of both proteins with a cellular imaging reader, as has been developed for example, but not exclusively, by Cellomics or EvotecOAI. In all cases the two or more binding partners can be different proteins with one being the protein of the invention, or in case of dimerization and/or oligomerization the protein of the invention itself. Proteins of the invention, for which one target mechanism of interest, but not the only one, would be such protein/protein interactions are fwd, Adk3, Gdh, or OSBP.

Of particular interest are screening assays for agents that have a low toxicity for mammalian cells. The term "agent" as used herein describes any molecule, e.g. protein or pharmaceutical, with the capability of altering or mimicking the physiological function of one or more of the proteins of the invention. Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise carbocyclic or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups.

Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, nucleic acids and derivatives, structural analogs or combinations thereof. Candidate agents are obtained from a wide variety of sources including libraries of synthetic

or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal.

Another technique for drug screening, which may be used, provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to the proteins of the invention large numbers of different small test compounds, e.g. aptamers, peptides, low-molecular weight compounds etc., are provided or synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with the proteins or fragments thereof, and washed. Bound proteins are then detected by methods well known in the art. Purified proteins can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support. In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding the protein specifically compete with a test compound for binding the protein. In this manner, the antibodies can be used to detect the presence of any peptide, which shares one or more antigenic determinants with the protein.

Finally, the invention also relates to a kit comprising at least one of

(a) a fwd, Adk3, Gdh, or OSBP nucleic acid molecule or a fragment thereof;

(b) a fwd, Adk3, Gdh, or OSBP amino acid molecule or a fragment or an isoform thereof;

(c) a vector comprising the nucleic acid of (a);

(d) a host cell comprising the nucleic acid of (a) or the vector of (b);

(e) a polypeptide encoded by the nucleic acid of (a);

(f) a fusion polypeptide encoded by the nucleic acid of (a);

(g) an antibody, an aptamer or another effector against the nucleic acid of (a) or the polypeptide of (b), (e) or (f) and

(h) an anti-sense oligonucleotide of the nucleic acid of (a).

The kit may be used for diagnostic or therapeutic purposes or for screening applications as described above. The kit may further contain user instructions.

The Figures show:

FIGURE 1 shows the content of energy storage triglyceride (TG) of Drosophila fwd (GadFly Accession Number CG7004) mutants. Shown is the change of triglyceride content of HD-EP(3)30148 flies caused by integration of the P-vector into the annotated transcription unit ('HD-EP30148 (90°C)', column 3) in comparison to controls containing about 2000 fly lines of the proprietary EP collection ('HD-control (90°C)', column 1,) and wildtype controls determined in more than 80 independent assays (referred to as 'WT-control (90°C)' column 2).

FIGURE 2 shows the molecular organization of the mutated fwd (GadFly Accession Number CG7004) gene locus.

FIGURE 3 shows the nucleic acid and amino acid sequences of the human phosphatidylinositol 4-kinase, catalytic, beta polypeptide (PIK4CB).
Figure 3A shows the nucleic acid sequence of human PIK4CB (SEQ ID NO: 1)
Figure 3B shows the amino acid sequence (one-letter code) of human PIK4CB (SEQ ID NO: 2).

FIGURE 4 shows the content of energy storage triglyceride (TG) of Drosophila Adk3 (GadFly Accession Number CG6612) mutants. Shown is the change of triglyceride content of HD-EP(3)36627 flies caused by integration of the P-vector into the annotated transcription unit ('HD-36627/TM3 (90°C)', column 3) in comparison to controls containing about 2000 fly lines of the proprietary EP collection ('HD-control (90°C)'), column 1,) and wildtype controls determined in more than 80 independent assays (referred to as 'WT-control (90°C)' column 2). Also shown is the change of triglyceride content of HD-EP(3)36627 flies at different assay conditions (70°C instead of 90°C) ('HD-36627/TM3 (70°C)', column 6) in comparison to controls containing about 880 fly lines of the proprietary EP collection ('HD-control (70°C)'), column 4,) and wildtype controls determined in 4 independent assays (referred to as 'WT-control (70°C)' column 5).

FIGURE 5 shows the molecular organization of the mutated Adk3 (GadFly Accession Number CG6612) gene locus.

FIGURE 6 shows the nucleic acid and amino acid sequences of the human adenylate kinase 3 (AK3) and adenylate kinase 3 alpha like (AKL3L).
Figure 6A shows the nucleic acid sequence of human AK3 (SEQ ID NO: 3)
Figure 6B shows the amino acid sequence (one-letter code) of human AK3 (SEQ ID NO: 4).
Figure 6C shows the nucleic acid sequence of human AKL3L (SEQ ID NO: 5)

Figure 6D shows the amino acid sequence (one-letter code) of human AKL3L (SEQ ID NO: 6).

FIGURE 7 shows the content of energy storage metabolites (ESM; triglyceride (TG) and glycogen) of Drosophila Gdh (GadFly Accession Number CG5320) mutants. Shown is the change of triglyceride content of HD-EP(3)35207 flies caused by integration of the P-vector into the annotated transcription unit ('HD-35207 (TG, 90°C)', column 3) in comparison to controls containing about 2000 fly lines of the proprietary EP collection ('HD-control (TG, 90°C)'), column 1,) and wildtype controls determined in more than 80 independent assays (referred to as 'WT-control (90°C)' column 2). Also shown is the change of glycogen content of HD-EP(3)35207 flies caused by integration of the P-vector the into the annotated transcription unit ('HD-35207 (glycogen, 90°C)', column 5) in comparison to controls (referred to as 'control (glycogen, 90°C)' column 4).

FIGURE 8 shows the molecular organization of the mutated Gdh (GadFly Accession Number CG5320) gene locus.

FIGURE 9 shows the nucleic acid and amino acid sequences of the human glutamate dehydrogenase 1 (GLUD1) and glutamate dehydrogenase 2 (GLUD2).

Figure 9A shows the nucleic acid sequence of human GLUD1 (SEQ ID NO: 7)

Figure 9B shows the amino acid sequence (one-letter code) of human GLUD1 (SEQ ID NO: 8).

Figure 9C shows the nucleic acid sequence of human GLUD2 (SEQ ID NO: 9)

Figure 9D shows the amino acid sequence (one-letter code) of human GLUD2 (SEQ ID NO: 10).

FIGURE 10 shows the content of energy storage triglyceride (TG) of Drosophila CG3860 (GadFly Accession Number) mutants. Shown is the change of triglyceride content of HD-EP(2)25831 flies caused by integration of the P-vector into the annotated transcription unit ('HD-EP25831 (90°C)', column 3) in comparison to controls containing about 2000 fly lines of the proprietary EP collection ('HD-control (90°C)'), column 1,) and wildtype controls determined in more than 80 independent assays (referred to as 'WT-control (90°C)' column 2).

FIGURE 11 shows the molecular organization of the mutated CG3860 (GadFly Accession Number) gene locus.

FIGURE 12 shows the nucleic acid and amino acid sequences of the human oxysterol binding protein-like 1A (OSBP1A) and 2 (OSBP2).

Figure 12A shows the nucleic acid sequence of human OSBP1A, transcript variant A (SEQ ID NO: 11)

Figure 12B shows the amino acid sequence (one-letter code) of human OSBP1A, isoform A (SEQ ID NO: 12).

Figure 12C shows the nucleic acid sequence of human OSBP1A, transcript variant B (SEQ ID NO: 13)

Figure 12D shows the amino acid sequence (one-letter code) of human OSBP1A, isoform B (SEQ ID NO: 14).

Figure 12E shows the nucleic acid sequence of human OSBP1A, transcript variant C (SEQ ID NO: 15)

Figure 12F shows the amino acid sequence (one-letter code) of human OSBP1A, isoform C (SEQ ID NO: 16).

Figure 12G shows the nucleic acid sequence of human OSBP2, transcript variant 1 (SEQ ID NO: 17)

Figure 12H shows the amino acid sequence (one-letter code) of human OSBP2, isoform 1 (SEQ ID NO: 18).

Figure 12I shows the nucleic acid sequence of human OSBP2, transcript variant 2 (SEQ ID NO: 19)

Figure 12J shows the amino acid sequence (one-letter code) of human OSB2, isoform 2 (SEQ ID NO: 20).

The examples illustrate the invention:

Example 1: Measurement of energy storage metabolites (ESM) contents in Drosophila

Mutant flies are obtained from a fly mutation stock collection. The flies are grown under standard conditions known to those skilled in the art. In the course of the experiment, additional feedings with bakers yeast (Saccharomyces cerevisiae) are provided for the EP-lines HD-EP(3)30148, HD-EP(3)35207, HD-EP(2)25831, and HD-EP(3)36627. The average change of triglyceride and glycogen (herein referred to as energy storage metabolites, ESM) content of Drosophila containing the EP-vector as homozygous viable or homozygous lethal integration was investigated in comparison to control flies, respectively (see FIGURES 1, 4, 7, and 10). For determination of ESM content, flies were incubated for 5 min at 70°C or 90°C in an aqueous buffer using a waterbath, followed by hot extraction. After another 5 min incubation at 70°C or 90°C and mild centrifugation, the triglyceride content of the flies extract was determined using Sigma Triglyceride (INT 336-10 or -20) assay by measuring changes in the optical density according to the manufacturer's protocol, and the glycogen content of the flies extract was determined using Roche (Starch UV-method Cat. No. 0207748) assay by measuring changes in the optical density according to the manufacturer's protocol. As a reference the protein content of the same extract was measured using BIO-RAD DC Protein Assay according to the manufacturer's protocol. These experiments and assays were repeated several times.

The average triglyceride level (µg triglyceride/µg protein) of 2108 fly lines of the proprietary EP-collection determined at 90°C (referred to as

'HD-control (90°C)' in FIGURES 1, 4, and 10, referred to as 'HD-control (TG, 90°C)' in FIGURE 7) is shown as 100% in the first column in FIGURES 1, 4, 7, and 10. The average triglyceride level of 883 fly lines of the proprietary EP-collection determined at 70°C (referred to as 'HD-control (70°C)' in FIGURE 4) is shown as 100% in the fourth column in FIGURE 4. The average triglyceride level (µg triglyceride/µg protein) of drosophila wildtype strain Oregon R flies determined in 84 independent assays at 90°C (referred to as 'WT-control (90°C)' in FIGURES 1, 4, 10, referred to as 'WT-control (TG, 90°C)' in FIGURE 7) is shown as 102% in the second column in FIGURES 1, 4, 7, and 10. The average triglyceride level of drosophila wildtype strain Oregon R flies determined in 4 independent assays at 70°C (referred to as 'WT-control (70°C)' in FIGURE 4) is shown as 116% in the fifth column in FIGURE 4. The average glycogen level (µg glycogen/µg protein) of an internal assay control consisting of two different wildtype strains and an inconspicuous EP-line of the HD stock collection (referred to as 'control (glycogen, 90°C)') is shown as 100% in the fourth column in FIGURE 7. Standard deviations of the measurements are shown as thin bars.

HD-EP(3)30148 homozygous flies show constantly a higher triglyceride content (µg triglyceride/µg protein) than the controls (column 3 in FIGURE 1, 'HD-EP30148 (90°C)'). Therefore, the loss of gene activity is responsible for changes in the metabolism of the energy storage triglycerides.

HD-EP(3)36627 heterozygous flies show constantly a higher triglyceride content (µg triglyceride/µg protein) than the controls (column 3 in FIGURE 4, 'HD-36627/TM3 (90°C)'). Therefore, the loss of gene activity is responsible for changes in the metabolism of the energy storage triglycerides.

HD-EP(3)35207 homozygous flies show constantly a lower triglyceride content (µg triglyceride/µg protein) than the controls (column 3 in FIGURE 7, 'HD-35207 (TG, 90°C)'). HD-EP(3)35207 homozygous flies also show a lower glycogen content (µg glycogen/µg protein) than the controls (column 5 in FIGURE 7, 'HD-35207 (glycogen, 90°C)'). Therefore, the loss of gene activity is responsible for changes in the metabolism of the energy storage metabolites.

HD-EP(2)25831 homozygous flies show constantly a higher triglyceride content (µg triglyceride/µg protein) than the controls (column 3 in FIGURE 10, 'HD-ep25831 (90°C)'). Therefore, the loss of gene activity is responsible for changes in the metabolism of the energy storage triglycerides.

Example 2: Identification of Drosophila genes responsible for changes in metabolite contents

Genomic DNA sequences were isolated that are localized directly adjacent to the EP vector (herein HD-EP(3)30148, HD-EP(3)35207, HD-EP(2)25831, and HD-EP(3)36627) integration.

Using those isolated genomic sequences public databases like Berkeley Drosophila Genome Project (GadFly) were screened thereby confirming the homozygous viable integration site of the HD-EP(3)30148 vector 122 base pairs 5prime of transcription variant CG7004-RA of the fwd gene in antisense orientation. FIGURE 2 shows the molecular organization of this gene locus. The chromosomal localization site of integration of the vector of HD-EP(3)30148 is at gene locus 3L, 61C1. In FIGURE 2, genomic DNA sequence is represented by the assembly as a black arrow in middle of the figure that includes the integration site of HD-EP(3)30148. Ticks represent the length in basepairs of the genomic DNA (1000 base pairs per tick).

Dark grey bars in the lower half of the figure, linked by dark grey lines represent cDNAs of the predicted genes (as predicted by the Berkeley Drosophila Genome Project). Predicted exons of the Drosophila cDNA of the gene fwd (GadFly Accession Number CG7004) are shown as dark grey bars and predicted introns as slim grey lines in the lower half of the figure and are labeled. The integation site of HD-EP(3)30148 is indicated with a black triangle 5prime of the first exon of the fwd predicted cDNA transcript variants. Therefore, expression of the cDNA encoding fwd could be affected by integration of the vector of line HD-EP(3)30148, leading to a change in the amount of energy storage triglycerides.

Using those isolated genomic sequences public databases like Berkeley Drosophila Genome Project (GadFly) were screened thereby confirming the heterozygous viable integration site of the HD-EP(3)36627 vector about 350 base pairs 5prime of Adk3 in sense orientation. FIGURE 2 shows the molecular organization of this gene locus. The chromosomal localization site of integration of the vector of HD-EP(3)36627 is at gene locus 3R, 86C7. In FIGURE 2, genomic DNA sequence is represented by the assembly as a black arrow in middle of the figure that includes the integration site of HD-EP(3)36627. Ticks represent the length in basepairs of the genomic DNA (1000 base pairs per tick). Dark grey bars in the lower half of the figure, linked by dark grey lines represent cDNAs of the predicted genes (as predicted by the Berkeley Drosophila Genome Project). Predicted exons of the Drosophila cDNA of the gene Adk3 (GadFly Accession Number CG6612) are shown as dark grey bars and predicted introns as slim grey lines in the lower half of the figure and are labeled. The integation site of HD-EP(3)36627 is indicated with a black triangle 5prime of the first exon of the Adk3 predicted cDNA transcript variants. Therefore, expression of the cDNA encoding Adk3 could be affected by integration of the vector of line HD-EP(3)36627, leading to a change in the amount of energy storage triglycerides.

Using those isolated genomic sequences public databases like Berkeley Drosophila Genome Project (GadFly) were screened thereby confirming the homozygous viable integration site of the HD-EP(3)35207 vector 62 base pairs 5prime of transcription variant CG7004-RA of the Gdh gene (Gadfly Accession Number CG5320) in sense orientation. FIGURE 2 shows the molecular organization of this gene locus. The chromosomal localization site of integration of the vector of HD-EP(3)35207 is at gene locus 3R, 95D1-4 (according to Flybase) or 3R, 95C5 (according to Gadfly). In FIGURE 2, genomic DNA sequence is represented by the assembly as a dotted grey line in middle of the figure that includes the integration site of HD-EP(3)35207. Numbers represent the coodinates of the genomic DNA (starting at position 19747173 on chromosome 3R, ending at position 19772173 on chromosome 3R). The insertion site of the P-element in Drosophila line HD-EP(3)35207 is shown as triangle in the "P Elements -" line and is labeled. Dark grey bars on the "cDNA +" and the "cDNA -" line, partly linked by light grey bars, represent the predicted genes (as predicted by the Berkeley Drosophila Genome Project, GadFly and by Magpie). Predicted exons are shown as dark grey bars, predicted introns are shown as light grey bars. The gene Gdh (Gacfly Accession Number CG5320) is labeled. Transcribed DNA sequences (ESTs) are shown as grey bars in the "EST +" and "EST -" lines. Therefore, expression of the cDNA encoding Gdh could be affected by integration of the vector of line HD-EP(3)35207, leading to a change in the amount of energy storage metabolites.

Using those isolated genomic sequences public databases like Berkeley Drosophila Genome Project (GadFly) were screened thereby confirming the homozygous viable integration site of the HD-EP(2)25831 vector into base pair3 of transcription variant CG3860-RA of the CG3860 gene in antisense orientation. FIGURE 2 shows the molecular organization of this gene locus. The chromosomal localization site of integration of the vector of HD-EP(2)25831 is at gene locus 2R, 60A16-B1. In FIGURE 2, genomic DNA sequence is represented by the assembly as a dotted grey line in

middle of the figure that includes the integration site of HD-EP(2)25831. Numbers represent the coodinates of the genomic DNA (starting at position 19035500 on chromosome 2R, ending at position 19039000 on chromosome 2R). The insertion site of the P-element in Drosophila line HD-EP(2)25831 is shown as arrow in the "P Elements +" line and is labeled. Dark grey bars onthe "cDNA +" and the "cDNA -" line, partly linked by light grey bars, represent the predicted genes (as predicted by the Berkeley Drosophila Genome Project, GadFly and by Magpie). Predicted exons are shown as dark grey bars, predicted introns are shown as light grey bars. The gene CG3860 (Gacfly Accession Number) is labeled. Transcribed DNA sequences (ESTs) are shown as grey bars in the "EST +" and "EST -" lines. Therefore, expression of the cDNA encoding CG3860 could be affected by integration of the vector of line HD-EP(2)25831, leading to a change in the amount of energy storage metabolites.

Table 1 is summarizing the data of our molecular analysis of the Drosophila proteins identified in this invention as being involved in the regulation of the metabolism.

Table 1. Molecular analysis of Drosophila fwd, Adk3, Gdh, or OSBP

Analysis	**Genetic interaction**
fwd	not described (Flybase)
Adk3	not described (Flybase)
Gdh	not described
CG3860	not described (Flybase)
Analysis	**Protein**
fwd	1-phosphatidylinositol 4-kinase (Flybase)
Adk3	adenylate kinase (Flybase)
Gdh	glutamate dehydrogenase (NAD(P)+) (Flybase)
CG3860	Oxysterol binding protein (Flybase)
Analysis	**Protein domains**
fwd	Phosphatidylinositol 3- and 4-kinase, Protein kinase-like (PK-like) (Flybase)
Adk3	Adenylate kinase (Flybase)
Gdh	Glutamate/leucine/phenylalanine/valinedehydrogenase; NAD(P)-binding Rossmann-fold domains; Aminoacid dehydrogenase-like
CG3860	Oxysterol binding protein (Flybase)
Analysis	**InterPro analysis**
fwd	Phosphatidylinositol 3- and 4-kinase (IPR000403)
Adk3	Adenylate kinase (IPR000850)
Gdh	not described (Flybase)
CG3860	Oxysterol binding protein (IPR000648)

Analysis	Locus
fwd	3L, 61C1 (Flybase); 3L, 61C1 (Gadfly release 3)
Adk3	3R, 86C7 (Flybase); 3R, 86C7 (Gadfly release 3)
Gdh	3R, 95D1-4 (FlyBase); 95C5 (GadFly)
CG3860	2R, 60A16-B1 (Flybase); 2R, 60A16-B1 (Gadfly release 3)
Analysis	**Ests**
fwd	several including LP07057
Adk3	few including GM21394
Gdh	
CG3860	several including GH12064
Analysis	**cDNA**
fwd	several including AF242375 (4464 base pairs mRNA, 2001; protein:AAK27793) & AY052049 (6341 base pairs mRNA, 2001; protein:AAK93473) (Flybase)
Adk3	AB050622 (651 base pairs mRNA, 2001; protein:BAB44152), AI945436 (537 base pairs mRNA, 2001) (Flybase)
Gdh	
CG3860	AI134566 (631 base pairs mRNA, 2001), AI945377 (530 base pairs mRNA, 2001), AI945437 (618 base pairs mRNA, 2001), AW940447 (518 base pairs mRNA, 2001), AY095008 (2102 base pairs mRNA, 2002; protein:AAM11336) (Flybase)
Analysis	**genomic DNA**
fwd	AE003467 (298640 base pairs DNA, 2000; protein:AAF47375) (Flybase)
Adk3	AE003689 (215984 base pairs DNA, 2000; protein:AAF54578) (Flybase)
Gdh	
CG3860	AE003462 (300542 base pairs DNA, 2000; protein:AAF47130) (Flybase)
Analysis	**NCBI locus ID**
fwd	45374, Dm fwd, four wheel drive, 61C1 Aliases: PI4K, CG7004, CT21674 RefSeq: NM_080083 Nucleotide: AE003467, AF242375, AI114377, AW944068, AY052049, BG632603, BG637878 Protein: AAF47375, AAK27793, AAK93473, NP_524822
Adk3	41318, Dm Adk3, Adenylate kinase-3, 86C7

	Aliases: AK3, DAK3, CG6612, CT20570, bs12h03.y1 RefSeq: NM_079588 Nucleotide: AE003689, AB050622 Protein: AAF54578, BAB44152, NP_524312
Gdh	42832, Dm Gdh, Glutamate dehydrogenase, 95C5 Aliases: GDH, gdh, DHE3, GLUD, Glud, GLU-D,CG5320, CT16932, GLUD pre-mRNA RefSeq: NM_079746 Nucleotide: AE003745, AQ025137, AQ254883, Z28976, Z28977, Z28978, Z28979, Z28980, Z29063, AW942692, AY061323, Y11314, Z29062 Protein: NP_524470, AAF56209, AAL28871, CAA72173, CAA82304
CG3860	37825, Dm CG3860, 60A16-60B1 Aliases: CT12843 RefSeq: NM_138021 Nucleotide: AE003462, AW940447, AY095008 Protein: AAF47130, AAM11336, NP_611865
Analysis	**Drosophila mutations & mutants**
fwd	There are 7 recorded alleles : 6 classical mutants (1 available from the public stock centers) and 1 wild-type (Flybase)
Adk3	not described (Flybase)
Gdh	not described (Flybase)
CG3860	not described (Flybase)
Analysis	**Phenotypic info**
fwd	Mutations of fwd affect the morphology and behaviour of the mitotic spindles of embryonic cleavage divisions to produce multipolar spindles in male meiosis and generate abnormal mitotic figures in larval neuroblasts. (Flybase)
Adk3	not described (Flybase)
Gdh	not described (Flybase)
CG3860	not described (Flybase)

Example 3: Identification of the human fwd, Adk3, Gdh, or OSBP homologous proteins

Fwd, Adk3, Gdh, or OSBP homologous proteins and nucleic acid molecules coding therefore are obtainable from insect or vertebrate species, e.g. mammals or birds. Particularly preferred are nucleic acids comprising Drosophila fwd, Adk3, Gdh, or OSBP or human fwd, Adk3, Gdh, or OSBP homologs. Sequences homologous to Drosophila fwd, Adk3, Gdh, or OSBP were identified using the publicly available program BLASTP 2.2.3 of the non-redundant protein data base of the National Center for Biotechnology Information (NCBI) (see, Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402). Table 2 shows the best human homologs of the Drosophila fwd, Adk3, Gdh, or OSBP genes.

The term "polynucleotide comprising the nucleotide sequence as shown in GenBank Accession number" relates to the expressible gene of the nucleotide sequences deposited under the corresponding GenBank Accession number. The term "GenBank Accession number" relates to NCBI GenBank database entries (Ref.: Benson et al., Nucleic Acids Res. 28 (2000) 15-18).

Table 2. Human homologous proteins to Drosophila fwd, Adk3, Gdh, or OSBP protein

I. fwd

NCBI (National Center for Biotechnology Information) human locus identification (ID): 5298, Hs PIK4CB, phosphatidylinositol 4-kinase, catalytic, beta polypeptide, 1q21

Aliases: PI4Kbeta, PI4K-BETA

OMIM: 602758

RefSeq: GenBank Accession Number NM_002651

Nucleotide: GenBank Accession Numbers AB005910, AJ011121, AJ011122, AJ011123, BC000029, U81802

Protein: GenBank Accession Numbers AAC51156, AAH00029, BAA21661, CAA09495, CAA09496, NP_002642

II. Adk3

NCBI (National Center for Biotechnology Information) human locus identification (ID): 205, Hs AK3, adenylate kinase 3, 1 (GTP:AMP phosphotransferase)

OMIM: 103030

RefSeq[R]: GenBank Accession Number NM_013410

Nucleotide: GenBank Accession Numbers BC016180, X60673

Protein: GenBank Accession Numbers AAH16180, CAA43088, NP_037542

NCBI (National Center for Biotechnology Information) human locus identification (ID): 50808, Hs AKL3L, adenylate kinase 3 alpha like, 9p24.1-p24.3

RefSeq: GenBank Accession Number NM_016282

Nucleotide: GenBank Accession Numbers AB021870, AK001553, AK001951, AK027534

Protein: GenBank Accession Numbers BAA87913, BAA91753, BAA91996, BAB55183, NP_057366

III. Gdh

NCBI (National Center for Biotechnology Information) human locus identification (ID): 2746, Hs GLUD1, glutamate dehydrogenase 1, 10q23.3

Aliases: GLUD

OMIM: 138130

RefSeq: GenBank Accession Number NM_005271

Nucleotide: GenBank Accession Numbers S60498, AK094782, J03248, M20867, M37154, X07674, X07769

Protein: GenBank Accession Numbers NP_005262, AAA52523, AAA52526, AAA52525, CAA30521, CAA30598

NCBI (National Center for Biotechnology Information) human locus identification (ID): 8307, Hs GLUD2, Glutamate dehydrogenase-2, Xq25

OMIM: 300144

RefSeq: GenBank Accession Number NM_012084

Nucleotide: GenBank Accession Numbers AC006144, U08997, X66310

Protein: GenBank Accession Numbers NP_036216, AAD05030, AAA20969, CAA46995

IV. CG3860

NCBI (National Center for Biotechnology Information) human locus identification (ID): 114876, Hs OSBPL1A, oxysterol binding protein-like 1A, 18q11.1

Aliases: ORP1, OSBPL1B, FLJ10217

OMIM: 606730

RefSeq[R]: GenBank Accession Numbers NM_018030, NM_080597, NM_133268

Nucleotide: GenBank Accession Numbers AF274714, AF323726, AF392449, AF392450, AK001079, AK021898, BC007004, BC022293

Protein: GenBank Accession Numbers AAG53407, AAH07004, AAK15154, AAL40662, AAL40663, BAA91496, NP_060500, NP_542164, NP_579802

NCBI (National Center for Biotechnology Information) human locus identification (ID): 9885, Hs OSBPL2, oxysterol binding protein-like 2, 20q13.3

Aliases: ORP2, ORP-2, MGC4307, MGC8342, FLJ20223, KIAA0772

OMIM: 606731

RefSeq[R]: GenBank Accession Numbers NM_014835, NM_144498

Nucleotide: GenBank Accession Numbers AL354836, AB018315, AF331963, AF392447, AK000230, AY028168, BC000296, BC004455, BC018812, none

Protein: GenBank Accession Numbers AAG53416, AAH00296, AAH04455, AAK18044, AAL40660, BAA34492, CAC22306, CAC22307, NP_055650, NP_653081, Q9H1P3

The mouse homologous cDNA encoding the polypeptides of the invention were identified as GenBank Accession Numbers XM_196305, XM_205921 (for the mouse homologs of PIK4CB), AB020239.1 (for the mouse homolog of Ak3), XM_129200.2 (for the mouse homolog of Akl3l), NM_008133 (for the mouse homolog of Gdh), NM_020573 (for the mouse homolog of Osbpl1a), and NM_144500 (for the mouse homolog of Osbpl2).

Example 4: Expression of the polypeptides in mammalian (mouse) tissues

To analyse the expression of the polypeptides disclosed in this invention in mammalian tissues, several mouse strains (preferably mice strains C57Bl/6J, C57Bl/6 ob/ob and C57Bl/KS db/db which are standard model systems in obesity and diabetes research) were purchased from Harlan Winkelmann (33178 Borchen, Germany) and maintained under constant temperature (preferably 22°C), 40 per cent humidity and a light / dark cycle of preferably 14 / 10 hours. The mice were fed a standard chow (for example, from ssniff Spezialitäten GmbH, order number ssniff M-Z V1126-000). For the fasting experiment ("fasted wild type mice"), wild type mice were starved for 48 h without food, but only water supplied ad libitum (see, for example, Schnetzler et al. J Clin Invest 1993 Jul;92(1):272-80, Mizuno et al. Proc Natl Acad Sci U S A 1996 Apr 16;93(8):3434-8). Animals were sacrificed at an age of 6 to 8 weeks. The animal tissues were isolated according to standard procedures known to those skilled in the art, snap frozen in liquid nitrogen and stored at -80°C until needed.

For analyzing the role of the proteins disclosed in this invention in the in vitro differentiation of different mammalian cell culture cells for the

conversion of pre-adipocytes to adipocytes, mammalian fibroblast (3T3-L1) cells (e.g., Green & Kehinde, Cell 1: 113-116, 1974) were obtained from the American Tissue Culture Collection (ATCC, Hanassas, VA, USA; ATCC- CL 173). 3T3-L1 cells were maintained as fibroblasts and differentiated into adipocytes as described in the prior art (e.g., Qiu. et al., J. Biol. Chem. 276:11988-95, 2001; Slieker et al., BBRC 251: 225-9, 1998). In brief, cells were plated in DMEM/10% FCS (Invitrogen, Karlsruhe, Germany) at 50,000 cells/well in duplicates in 6-well plastic dishes and cultured in a humidified atmosphere of 5% CO_2 at 37°C. At confluence (defined as day 0: d0) cells were transferred to serum-free (SF) medium, containing DMEM/HamF12 (3:1; Invitrogen), fetuin (300 µg/ml; Sigma, Munich, Germany), transferrin (2 µg/ml; Sigma), pantothenate (17 µM; Sigma), biotin (1 µM; Sigma), and EGF (0.8nM; Hoffmann-La Roche, Basel, Switzerland). Differentiation was induced by adding dexamethasone (DEX; 1 µM; Sigma), 3-methyl-isobutyl-1-methylxanthine (MIX; 0.5mM; Sigma), and bovine insulin (5 µg/ml; Invitrogen). Four days after confluence (d4), cells were kept in SF medium, containing bovine insulin (5 µg/ml) until differentiation was completed. At various time points of the differentiation procedure, beginning with day 0 (day of confluence) and day 2 (hormone addition; for example, dexamethasone and 3-isobutyl-1-methylxanthine), up to 10 days of differentiation, suitable aliquots of cells were taken every two days.

RNA was isolated from mouse tissues or cell culture cells using Trizol Reagent (for example, from Invitrogen, Karlsruhe, Germany) and further purified with the RNeasy Kit (for example, from Qiagen, Germany) in combination with an DNase-treatment according to the instructions of the manufacturers and as known to those skilled in the art. Total RNA was reverse transcribed (preferably using Superscript II RNaseH- Reverse Transcriptase, from Invitrogen, Karlsruhe, Germany) and subjected to Taqman analysis preferably using the Taqman 2xPCR Master Mix (from Applied Biosystems, Weiterstadt, Germany; the Mix contains according to

the Manufacturer for example AmpliTaq Gold DNA Polymerase, AmpErase UNG, dNTPs with dUTP, passive reference Rox and optimized buffer components) on a GeneAmp 5700 Sequence Detection System (from Applied Biosystems, Weiterstadt, Germany).

Example 5: In vitro assays for the determination of triglyceride and glycogen storage

Obesity is known to be caused by different reasons such as non-insulin dependent diabetes, increase in triglycerides, increase in carbohydrate bound energy and low energy expenditure. For example, an increase in energy expenditure (and thus, lowering the body weight) would include the elevated utilization of both circulating and intracellular glucose and triglycerides, free or stored as glycogen or lipids as fuel for energy and/or heat production. In this invention, we therefore show the cellular level of triglycerides and glycogen in cells overexpressing the protein of the invention.

Retroviral infection of preadipocytes

Packaging cells were transfected with retroviral plasmids pLPCX carrying the mouse transgene encoding a protein of the invention and a selection marker using calcium phosphate procedure. Control cells were infected with pLPCX carrying no transgene. Briefly, exponentially growing packaging cells were seeded at a density of 350,000 cells per 6-well in 2 ml DMEM + 10 % FCS one day before transfection. 10 min before transfection chloroquine was added directly to the overlying medium (25 μM end concentration). A 250 μl transfection mix consisting of 5 μg plasmid-DNA (candidate:helper-virus in a 1:1 ratio) and 250 mM $CaCl_2$ was prepared in a 15 ml plastic tube. The same volume of 2 x HBS (280 μM NaCl, 50 μM HEPES, 1.5 mM Na_2HPO_4, pH 7.06) was added and air bubbles were injected into the mixture for 15 sec. The transfection mix

was added drop wise to the packaging cells, distributed and the cells were incubated at 37°C, 5 % CO_2 for 6 hours. The cells were washed with PBS and the medium was exchanged with 2 ml DMEM + 10 % CS per 6-well. One day after transfection the cells were washed again and incubated for 2 days of virus collection in 1 ml DMEM + 10 % CS per 6-well at 32°C, 5 % CO_2. The supernatant was then filtered through a 0.45 µm cellulose acetate filter and polybrene (end concentration 8 µg/ml) was added. Mammalian fibroblast (3T3-L1) cells in a sub-confluent state were overlaid with the prepared virus containing medium. The infected cells were selected for 1 week with 2 µg/ml puromycin. Following selection the cells were checked for transgene expression by western blot and immunofluorescence. Overexpressing cells were seeded for differentiation.

3T3-L1 cells were maintained as fibroblasts and differentiated into adipocytes as described in the prior art and supra. For analysing the role of the proteins disclosed in this invention in the in vitro assays for the determination of triglyceride storage, synthesis and transport were performed.

Preparation of cell lysates for analysis of metabolites

Starting at confluence (d0), cell media was changed every 48 hours. Cells and media were harvested 8 hours prior to media change as follows. Media was collected, and cells were washed twice in PBS prior to lyses in 600 microl HB-buffer (0.5% polyoxyethylene 10 tridecylethane, 1 mM EDTA, 0.01M NaH_2PO_4, pH 7.4). After inactivation at 70°C for 5 minutes, cell lysates were prepared on Bio 101 systems lysing matrix B (0.1 mm silica beads; Q-Biogene, Carlsbad, USA) by agitation for 2 x 45 seconds at a speed of 4.5 (Fastprep FP120, Bio 101 Thermosavant, Holbrock, USA). Supernatants of lysed cells were collected after centrifugation at 3000 rpm for 2 minutes, and stored in aliquots for later analysis at -80°C.

Changes in cellular triglyceride levels during adipogenesis

Cell lysates and media were simultaneously analysed in 96-well plates for total protein and triglyceride content using the Bio-Rad DC Protein assay reagent (Bio-Rad, Munich, Germany) according to the manufacturer's instructions and a modified enzymatic triglyceride kit (GPO-Trinder; Sigma) briefly final volumes of reagents were adjusted to the 96-well format as follows: 10 µl sample was incubated with 200 µl reagent A for 5 minutes at 37°C. After determination of glycerol (initial absorbance at 540 nm), 50 µl reagent B was added followed by another incubation for 5 minutes at 37°C (final absorbance at 540 nm). Glycerol and triglyceride concentrations were calculated using a glycerol standard set (Sigma) for the standard curve included in each assay.

Changes in cellular glycogen levels during adipogenesis

Cell lysates and media were simultaneously analysed in triplicates in 96-well plates for total protein and glycogen content using the Bio-Rad DC Protein assay reagent (Bio-Rad, Munich, Germany) according to the manufacturer's instructions and an enzymatic starch kit from Hoffmann-La Roche (Basel, Switzerland). 10-µl samples were incubated with 20-µl amyloglucosidase solution for 15 minutes at 60°C to digest glycogen to glucose. The glucose is further metabolised with 100 µl distilled water and 100 µl of enzyme cofactor buffer and 12 µl of enzyme buffer (hexokinase and glucose phosphate dehydrogenase). Background glucose levels are determined by subtracting values from a duplicate plate without the amyloglucosidase. Final absorbance is determined at 340 nm. HB-buffer as blank, and a standard curve of glycogen (Hoffmann-La Roche) were included in each assay. Glycogen content in samples were calculated using a standard curve.

Synthesis of lipids during adipogenesis

During the terminal stage of adipogenesis (day 12) cells were analysed for their ability to metabolise lipids. A modified protocol to the method of

Jensen et al (2000) for lipid synthesis was established. Cells were washed 3 times with PBS prior to serum starvation in Krebs-Ringer-Bicarbonate-Hepes buffer (KRBH; 134 nM NaCl, 3.5 mM KCl, 1.2 mM KH_2PO_4, 0.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 5 mM $NaHCO_3$, 10 mM Hepes, pH 7.4), supplemented with 0.1% FCS for 2.5h at 37°C. For insulin-stimulated lipid synthesis, cells were incubated with 1 μM bovine insulin (Sigma; carrier: 0.005N HCl) for 45 min at 37°C. Basal lipid synthesis was determined with carrier only. ^{14}C(U)-D-Glucose (NEN Life Sciences) in a final activity of 1μCi/Well/ml in the presence of 5 mM glucose was added for 30 min at 37°C. For the calculation of background radioactivity, 25 μM cytochalasin B (Sigma) was used. All assays were performed in duplicate wells. To terminate the reaction, cells were washed 3 times with ice cold PBS, and lysed in 1 ml 0.1N NaOH. Protein concentration of each well was assessed using the standard Biuret method (Protein assay reagent; Bio-Rad). Total lipids were separated from aqueous phase after overnight extraction in Insta-Fluor scintillation cocktail (Packard Bioscience) followed by scintillation counting.

Transport and metabolism of free fatty acids during adipogenesis

During the terminal stage of adipogenesis (d12) cells were analysed for their ability to transport long chain fatty acid across the plasma membrane. A modified protocol to the method of Abumrad et al (1991) (Proc. Natl. Acad. Sci. USA, 1991: 88; 6008-12) for cellular transportation of fatty acid was established. In summary, cells were washed 3 times with PBS prior to serum starvation. This was followed by incubation in KRBH buffer supplemented with 0.1% FCS for 2.5h at 37°C. Uptake of exogenous free fatty acids was initiated by the addition of isotopic media containing non radioactive oleate and (^{3}H)oleate (NEN Life Sciences) complexed to serum albumin in a final activity of 1μCi/Well/ml in the presence of 5 mM glucose for 30min at room temperature (RT). For the calculation of passive diffusion (PD) in the absence of active transport (AT) across the plasma membrane 20mM of phloretin in glucose free media (Sigma) was added for

30 min at RT. All assays were performed in duplicate wells. To terminate the active transport 20mM of phloretin in glucose free media was added to the cells. Cells were lysed in 1 ml 0.1N NaOH and the protein concentration of each well were assessed using the standard Biuret method (Protein assay reagent; Bio-Rad). Esterified fatty acids were separated from free fatty acids using overnight extraction in Insta-Fluor scintillation cocktail (Packard Bioscience) followed by scintillation counting.

Example 6: Glucose uptake assay

For the determination of glucose uptake, cells were washed 3 times with PBS prior to serum starvation in KRBH buffer supplemented with 0.1% FCS and 0.5mM Glucose for 2.5h at 37°C. For insulin-stimulated glucose uptake, cells were incubated with 1 microM bovine insulin (Sigma; carrier: 0.005N HCl) for 45 min at 37°C. Basal glucose uptake was determined with carrier only. Non-metabolizable 2-deoxy-^{3}H-D-glucose (NEN Life Science, Boston, USA) in a final activity of 0,4 μCi/Well/ml was added for 30 min at 37°C. For the calculation of background radioactivity, 25 μM cytochalasin B (Sigma) was used. All assays were performed in duplicate wells. To terminate the reaction, cells were washed 3 times with ice cold PBS, and lysed in 1 ml 0.1N NaOH. Protein concentration of each well was assessed using the standard Biuret method (Protein assay reagent; Bio-Rad), and scintillation counting of cell lysates in 10 volumes Ultima-gold cocktail (Packard Bioscience, Groningen, Netherlands) was performed.

Example 7: Generation and analysis of Pik4cb, Ak3, Akl3l, Glud, Osbpl1a, or Osbpl2 transgenic mice

Generation of the transgenic animals

Mouse Pik4cb, Ak3, Akl3l, Glud, Osbpl1a, or Osbpl2 cDNA was isolated from mouse brown adipose tissue (BAT) using standard protocols as

known to those skilled in the art. The cDNA was amplified by RT-PCR and point mutations were introduced into the cDNA.

The resulting mutated cDNA was cloned into a suitable transgenic expression vector. The transgene was microinjected into the male pronucleus of fertilized mouse embryos (preferably strain C57/BL6/CBA F1 (Harlan Winkelmann). Injected embryos were transferred into pseudo-pregnant foster mice. Transgenic founders were detected by PCR analysis. Two independent transgenic mouse lines containing the construct were established and kept on a C57/BL6 background. Briefly, founder animals were backcrossed with C57/BL6 mice to generate F1 mice for analysis. Transgenic mice were continously bred onto the C57/Bl6 background. The expression of the proteins of the invention can be analyzed by taqman analysis as described above, and further analysis of the mice can be done as known to those skilled in the art.

Claims

1. A pharmaceutical composition comprising a nucleic acid molecule encoding fwd, Adk3, Gdh, or OSBP or a homologue thereof or a polypeptide encoded thereby or encoded by a fragment or a variant of said nucleic acid molecule or said polypeptide or an effector of said nucleic acid molecule or said polypeptide, preferably together with pharmaceutically acceptable carriers and diluents.

2. The composition of claim 1, wherein the nucleic acid molecule is a vertebrate or insect fwd, Adk3, Gdh, or OSBP nucleic acid, particulary encoding the human fwd, Adk3, Gdh, or OSBP homologs (such as human phosphatidylinositol 4-kinase, catalytic, beta polypeptide, adenylate kinase 3, adenylate kinase 3 alpha like, glutamate dehydrogenase 1, glutamate dehydrogenase 2, oxysterol binding protein-like 1A, and oxysterol binding protein-like 2), and/or a nucleic molecule which is complementary thereto or a fragment thereof or a variant thereof.

3. The composition of claim 1 or 2, wherein said nucleic acid molecule is selected from the group consisting of

   (a) a nucleic acid molecule encoding a polypeptide as deposited under GenBank Accession Number NM_002651, NM_013410, NM_016282, NM_005271, NM_012084, NM_018030, NM_080597, NM_133268, NM_014835, NM_144498 or an isoform, fragment or variant of the polypeptide as deposited under GenBank Accession Number NP_002642, NP_037542, NP_057366, NP_005262, NP_036216, NP_060500, NP_542164, NP_579802, NP_055650, NP_653081;

(b) a nucleic acid molecule which comprises or is the nucleic acid molecule as deposited under GenBank Accession Number NM_002651, NM_013410, NM_016282, NM_005271, NM_012084, NM_018030, NM_080597, NM_133268, NM_014835, NM_144498,

(c) a nucleic acid molecule being degenerate with as a result of the genetic code to the nucleic acid sequences as defined in (a) or (b),

(d) a nucleic acid molecule that hybridizes at 50°C in a solution containing 1 x SSC and 0.1% SDS to a nucleic acid molecule as defined in claim 2 or as defined in (a) to (c) and/or a nucleic acid molecule which is complementary thereto;

(e) a nucleic acid molecule that encodes a polypeptide which is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably at least 98% and up to 99,6% identical to the human phosphatidylinositol 4-kinase, catalytic, beta polypeptide, adenylate kinase 3, adenylate kinase 3 alpha like, glutamate dehydrogenase 1, glutamate dehydrogenase 2, oxysterol binding protein-like 1A, and oxysterol binding protein-like 2 variants, as defined in claim 2 or to a polypeptide as defined in (a);

(f) a nucleic acid molecule that differs from the nucleic acid molecule of (a) to (e) by mutation and wherein said mutation causes an alteration, deletion, duplication or premature stop in the encoded polypeptide.

4. The composition of any one of claims 1-3, wherein the nucleic acid molecule is a DNA molecule, particularly a cDNA or a genomic DNA.

5. The composition of any one of claims 1-4, wherein said nucleic acid encodes a polypeptide contributing to regulating the energy homeostasis and/or the metabolism of triglycerides.

6. The composition of any one of claims 1-5, wherein said nucleic acid molecule is a recombinant nucleic acid molecule.

7. The composition of any one of claims 1-6, wherein the nucleic acid molecule is a vector, particularly an expression vector.

8. The composition of any one of claims 1-5, wherein the polypeptide is a recombinant polypeptide.

9. The composition of claim 8, wherein said recombinant polypeptide is a fusion polypeptide.

10. The composition of any one of claims 1-7, wherein said nucleic acid molecule is selected from hybridization probes, primers and anti-sense oligonucleotides.

11. The composition of any one of claims 1-10 which is a diagnostic composition.

12. The composition of any one of claims 1-10 which is a therapeutic composition.

13. The composition of any one of claims 1-12 for the manufacture of an agent for detecting and/or verifying, for the treatment, alleviation and/or prevention of metabolic diseases or dysfunctions, for example, but not limited to, metabolic syndrome including obesity, diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones, and others, in cells, cell masses, organs and/or subjects.

14. Use of a nucleic acid molecule of the fwd, Adk3, Gdh, or OSBP gene family or a polypeptide encoded thereby or a fragment or a

variant of said nucleic acid molecule or said polypeptide or an effector of said nucleic or polypeptide for controlling the function of a gene and/or a gene product which is influenced and/or modified by a fwd, Adk3, Gdh, or OSBP homologous polypeptide.

15. Use of the nucleic acid molecule of the fwd, Adk3, Gdh, or OSBP gene family or use of a nucleic acid molecule encoding fwd, Adk3, Gdh, or OSBP or a homologue thereof or use of a polypeptide encoded thereby, or use of a fragment or a variant of said nucleic acid molecule or said polypeptide, or use of an effector of said nucleic acid molecule or said polypeptide for identifying substances capable of interacting with a fwd, Adk3, Gdh, or OSBP homologous polypeptide.

16. A non-human transgenic animal exhibiting a modified expression of a fwd, Adk3, Gdh, or OSBP homologous polypeptide.

17. The animal of claim 16, wherein the expression of the fwd, Adk3, Gdh, or OSBP homologous polypeptide is increased and/or reduced.

18. A recombinant host cell exhibiting a modified expression of a fwd, Adk3, Gdh, or OSBP homologous polypeptide, or a recombinant host cell which comprises a nucleic acid molecule as defined in any one of claims 1 to 6.

19. The cell of claim 18 which is a human cell.

20. A method of identifying a (poly)peptide involved in the regulation of energy homeostasis and/or metabolism of triglycerides in a mammal comprising the steps of

(a) contacting a collection of (poly)peptides with a fwd, Adk3, Gdh, or OSBP homologous polypeptide or a fragment thereof under conditions that allow binding of said (poly)peptides;

(b) removing (poly)peptides which do not bind and

(c) identifying (poly)peptides that bind to said fwd, Adk3, Gdh, or OSBP homologous polypeptide.

21. A method of screening for an agent which modulates the interaction of a fwd, Adk3, Gdh, or OSBP homologous polypeptide with a binding target/agent, comprising the steps of

(a) incubating a mixture comprising

(aa) a fwd, Adk3, Gdh, or OSBP homologous polypeptide or a fragment thereof;

(ab) a binding target/agent of said fwd, Adk3, Gdh, or OSBP homologous polypeptide or fragment thereof; and

(ac) a candidate agent

under conditions whereby said fwd, Adk3, Gdh, or OSBP homologous polypeptide or fragment thereof specifically binds to said binding target/agent at a reference affinity;

(b) detecting the binding affinity of said fwd, Adk3, Gdh, or OSBP homologous polypeptide or fragment thereof to said binding target to determine a (candidate) agent-biased affinity; and

(c) determining a difference between (candidate) agent-biased affinity and reference affinity.

22. A method for screening for an agent, which modulates the activity of a fwd, Adk3, Gdh, or OSBP homlogous polypeptide, comprising the steps of

(a) incubating a mixture comprising

(aa) a fwd, Adk3, Gdh, or OSBP homologous polypeptide or a fragment thereof; and

(ab) a candidate agent

under conditions whereby said fwd, Adk3, Gdh, or OSBP homologous polypeptide or fragment thereof exhibits a reference activity,

(b) detecting the activity of said fwd, Adk3, Gdh, or OSBP homologous polypeptide or fragment thereof to determine a (candidate) agent-biased activity; and

(c) determining a difference between (candidate) agent-biased activity and reference activity.

23. A method of producing a composition comprising the (poly)peptide identified by the method of claim 20 or the agent identified by the method of claim 21 or 22 with a pharmaceutically acceptable carrier, diluent and/or adjuvant.

24. The method of claim 23 wherein said composition is a pharmaceutical composition for preventing, alleviating or treating of diseases and disorders, including metabolic diseases or dysfunctions, for example, but not limited to, such as metabolic syndrome including obesity, diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hyper-cholesterolemia (dyslipidemia), and gallstones, and other diseases and disorders.

25. Use of a (poly)peptide as identified by the method of claim 20 or of an agent as identified by the method of claim 21 or 22 for the preparation of a pharmaceutical composition for the treatment, alleviation and/or prevention of of diseases and disorders, including metabolic diseases or dysfunctions, for example, but not limited to, metabolic syndrome including obesity, diabetes mellitus, eating

disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones, and other diseases and disorders.

26. Use of a nucleic acid molecule as defined in any one of claims 1 to 6 or 10, use of a polypeptide as defined in any one of claims 1 to 6, 8 or 9, use of a vector as defined in claim 7, use of a host cell as defined in claim 18 or 19 for the preparation of a pharmaceutical composition for the treatment, alleviation and/or prevention of of diseases and disorders, including metabolic diseases or dysfunctions, for example, but not limited to, metabolic syndrome including obesity, diabetes mellitus, eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia (dyslipidemia), and gallstones, and other diseases and disorders.

27. Use of a nucleic acid molecule of the fwd, Adk3, Gdh, or OSBP gene family or of a fragment thereof for the preparation of a non-human animal which over- or under-expresses the fwd, Adk3, Gdh, or OSBP gene product.

28. Kit comprising at least one of

(a) a fwd, Adk3, Gdh, or OSBP nucleic acid molecule or a fragment or an isoform thereof;

(b) a fwd, Adk3, Gdh, or OSBP amino acid molecule or a fragment or an isoform thereof;

(c) a vector comprising the nucleic acid of (a);

(d) a host cell comprising the nucleic acid of (a) or the vector of (b);

(e) a polypeptide encoded by the nucleic acid of (a), expressed by the vector of (c) or the host cell of (a);

(f) a fusion polypeptide encoded by the nucleic acid of (a);

(g) an antibody, an aptamer or another effector against the nucleic acid of (a) or the polypeptide of (b) , (e) , or (f) and /or

(h) an anti-sense oligonucleotide of the nucleic acid of (a).

Abstract

The present invention discloses fwd, Adk3, Gdh, or OSBP homologous proteins regulating the energy homeostasis and the metabolism of triglycerides, and polynucleotides, which identify and encode the proteins disclosed in this invention. The invention also relates to the use of these sequences in the diagnosis, study, prevention, and treatment of metabolic diseases and disorders.

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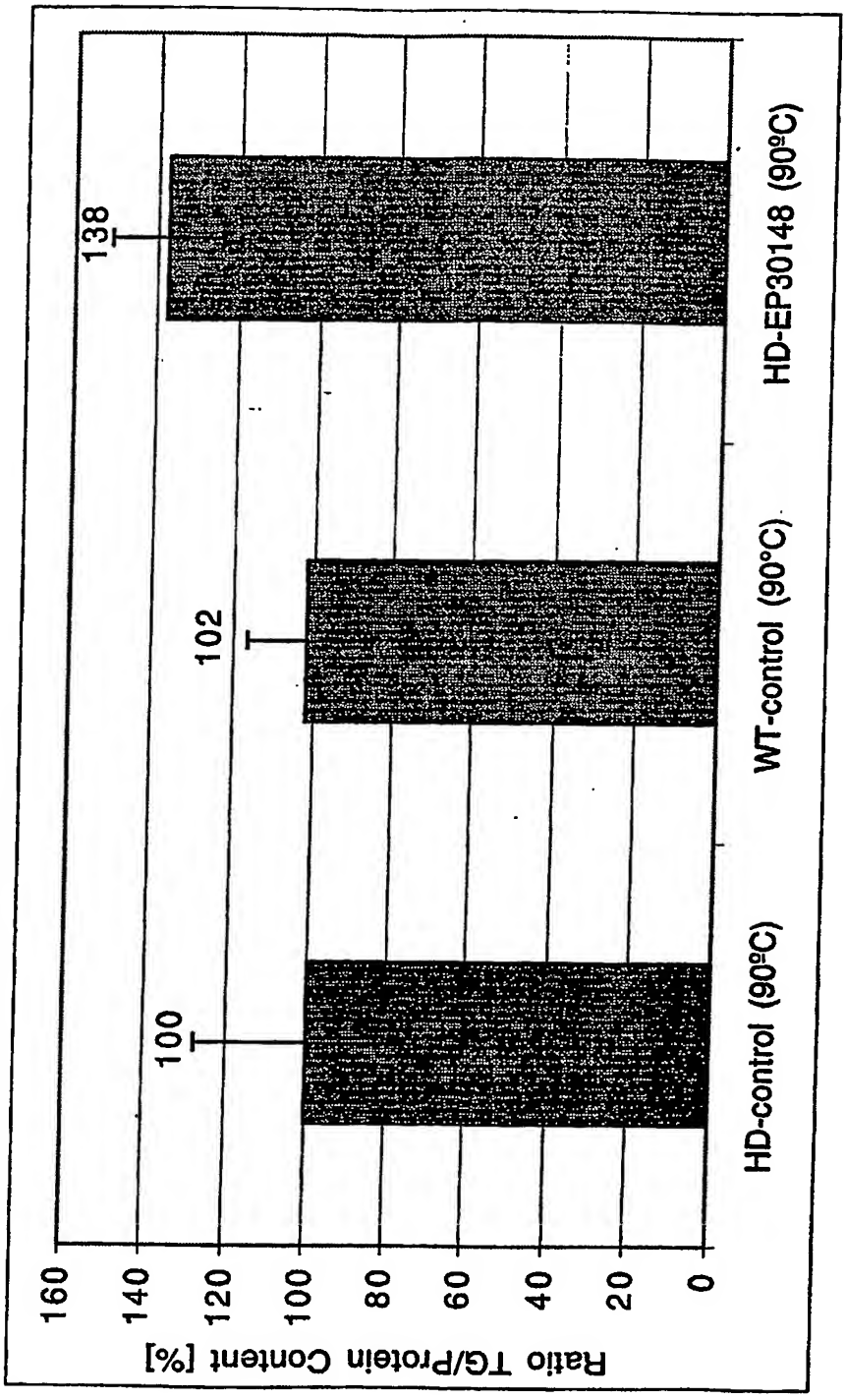

FIGURE 1. Energy storage metabolite content of a Drosophila fwd (Gadfly Acc. No. CG7004) mutant

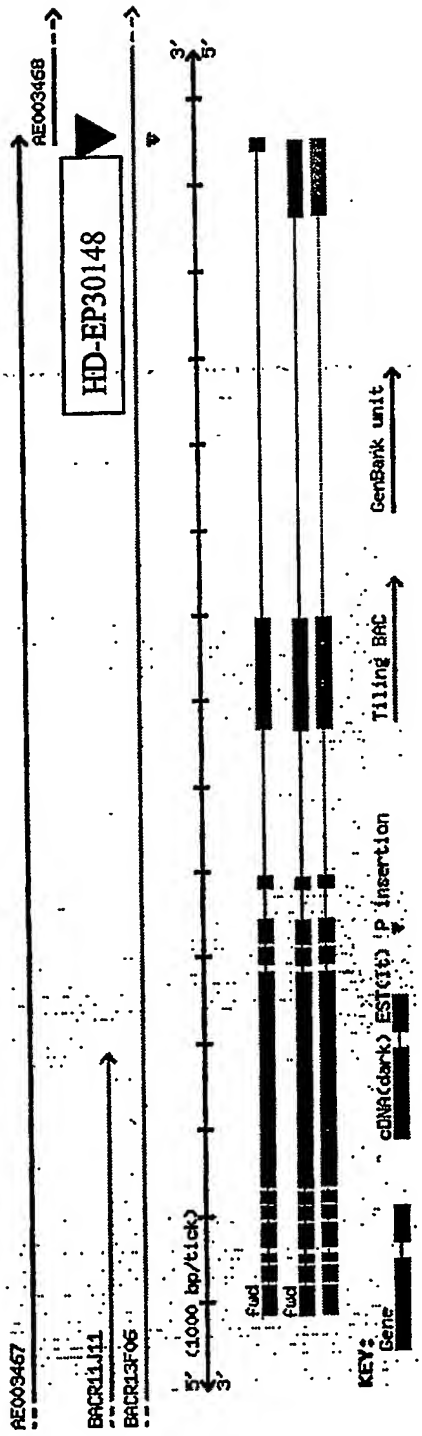

Figure 2. Molecular organization of the fwd gene (GadFly Acession Number CG7004)

FIGURE 3. Nucleic acid sequences and amino acid sequences of the human phosphatidylinositol 4-kinase, catalytic, beta polypeptide

FIGURE 3A. Homo sapiens phosphatidylinositol 4-kinase, catalytic, beta polypeptide (PIK4CB), Nucleic acid sequence (SEQ ID NO: 1)

```
   1 cagattacac ttggttgact actccggagc agccactaag agggatgaac aggcctgcgt
  61 ggaaattgaa tgagattctt ggaagctcga agtctggctg tggccatggg agatacagta
 121 gtggagcctg ccccttgaa gccaacttct gagcccactt ctggcccacc agggaataat
 181 gggggtccc tgctaagtgt catcacggag ggggtcgggg aactatcagt gattgaccct
 241 gaggtggccc agaaggcctg ccaggaggtg ttggagaaag tcaagctttt gcatggaggc
 301 gtggcagtct ctagcagagg caccccactg gagttggtca atggggatgg tgtggacagt
 361 gagatccgtt gcctagatga tccacctgcc cagatcaggg aggaggaaga tgagatgggg
 421 gccgctgtgg cctcaggcac agccaaagga gcaagaagac ggcggcagaa caactcagct
 481 aaacagtctt ggctgctgag gctgtttgag tcaaaactgt ttgacatctc catggccatt
 541 tcatacctgt ataactccaa ggagcctgga gtacaagcct acattggcaa ccggctcttc
 601 tgctttcgca acgaggacgt ggacttctat ctgccccagt tgcttaacat gtacatccac
 661 atggatgagg acgtgggtga tgccattaag ccctacatag tccaccgttg ccgccagagc
 721 attaactttt ccctccagtg tgccctgttg cttggggcct attcttcaga catgcacatt
 781 tccactcaac gacactcccg tgggaccaag ctacggaagc tgatcctctc agatgagcta
 841 aagccagctc acaggaagag ggagctgccc tccttgagcc cggcccctga cacagggctg
 901 tctccctcca aaaggactca ccagcgctct aagtcagatg ccactgccag cataagtctc
 961 agcagcaacc tgaaacgaac agccagcaac cctaaagtgg agaatgagga tgaggagctc
1021 tcctccagca ccgagagtat tgataattca ttcagttccc ctgttcgact ggctcctgag
1081 agagaattca tcaagtccct gatggcgatc ggcaagcggc tggccacgct ccccaccaaa
1141 gagcagaaaa cacagaggct gatctcagag ctctccctgc tcaaccataa gctccctgcc
1201 cgagtctggc tgcccactgc tggctttgac caccacgtgg tccgtgtacc ccacacacag
1261 gctgttgtcc tcaactccaa ggacaaggct ccctacctga tttatgtgga agtccttgaa
1321 tgtgaaaact ttgacaccac cagtgtccct gcccggatcc ccgagaaccg aattcggagt
1381 acgaggtccg tagaaaactt gcccgaatgt ggtattaccc atgagcagcg agctggcagc
1441 ttcagcactg tgcccaacta tgacaacgat gatgaggcct ggtcggtgga tgacataggc
1501 gagctgcaag tggagctccc cgaagtgcat accaacagct gtgacaacat ctcccagttc
1561 tctgtggaca gcatcaccag ccaggagagc aaggagcctg tgttcattgc agcaggggac
1621 atccgccggc gcctttcgga acagctggct catacccccga cagccttcaa acgagaccca
1681 gaagatcctt ctgcagttgc tctcaaagag ccctggcagg agaaagtacg gcggatcaga
1741 gagggctccc cctacggcca tctccccaat tggcggctcc tgtcagtcat tgtcaagtgt
1801 ggggatgacc ttcggcaaga gcttctggcc tttcaggtgt tgaagcaact gcagtccatt
1861 tgggaacagg agcgagtgcc cctttggatc aagccataca agattcttgt gatttcggct
1921 gatagtggca tgattgaacc agtggtcaat gctgtgtcca tccatcaggt gaagaaacag
1981 tcacagctct ccttgctcga ttacttccta caggagcacg gcagttacac cactgaggca
2041 ttcctcagtg cacagcgcaa ttttgtgcaa agttgtgctg ggtactgctt ggtctgctac
2101 ctgctgcaag tcaaggacag acacaatggg aatatccttt tggacgcaga aggccacatc
2161 atccacatcg actttggctt catcctctcc agctcacccc gaaatctggg ctttgagacg
2221 tcagccttta agctgaccac agagtttgtg gatgtgatgg gcggcctgga tggcgacatg
2281 ttcaactact ataagatgct gatgctgcaa gggctgattg ccgctcggaa acacatggac
2341 aaggtggtgc agatcgtgga gatcatgcag caaggttctc agcttccttg cttccatggc
2401 tccagcacca ttcgaaacct caaagagagg ttccacatga gcatgactga ggagcagctg
2461 cagctgctgg tggagcagat ggtggatggc agtatgcggt ctatcaccac caaactctat
2521 gacggcttcc agtacctcac caacggcatc atgtgacacg ctcctcagcc caggagtggt
2581 gggggtccg gggcaccctc cctagagggc ccttgtctga gaaaccccaa accaggaaac
2641 cccacctacc caaccatcca cccaagggaa atggaaggca agaaacacga aggatcatgt
2701 ggtaactgcg agagcttgct gaggggtggg agagccagct gtggggtcca gacttgttgg
2761 ggcttccctg cccctcctgg tctgtgtcag tattaccacc agactgactc caggactcac
2821 tgccctccag aaaacagagg tgacaaatgt gagggacact ggggcctttc ttctccttgt
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2941 agtggggaag agtaggttct cggtacttag gacttgatcc tgtggttggc cactggccat
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3181 ggaattccct accctgactc cttccccaaa cccagggaaa agagctctca attttttatt
3241 tttaattttt gtttgaaata aagtccttag ttagccactt gtgtcatttc caggttttct
3301 gggggagtgc agggggagat gggtgatgag gtatgaacgg atgcctcagt gtccaagata
3361 caaaaggcac cacatagaag tttgcttttt ccctgcctgt cttggtcact accacctctt
3421 ccctgagaag ggcgggcctt ccatgttctc tcacccgctt caactccaca t
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FIGURE 3B. Homo sapiens phosphatidylinositol 4-kinase, catalytic, beta polypeptide (PIK4CB), Amino acid sequence (SEQ ID NO: 2)

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   1 mrflearsla vamgdtvvep aplkptsept sgppgnnggs llsvitegvg elsvidpeva
  61 qkacqevlek vkllhggvav ssrgtplelv ngdgvdseir clddppaqir eeedemgaav
 121 asgtakgarr rrqnnsakqs wllrlfeskl fdismaisyl ynskepgvqa yignrlfcfr
 181 nedvdfylpq llnmyihmde dvgdaikpyi vhrcrqsinf slqcalllga yssdmhistq
 241 rhsrgtklrk lilsdelkpa hrkrelpsls papdtglsps krthqrsksd atasislssn
 301 lkrtasnpkv enedeelsss tesidnsfss pvrlaperef ikslmaigkr latlptkeqk
 361 tqrliselsl lnhklparvw lptagfdhhv vrvphtqavv lnskdkapyl iyvevlecen
 421 fdttsvpari penrirstrs venlpecgit heqragsfst vpnydnddea wsvddigelq
 481 velpevhtns cdnisqfsvd sitsqeskep vfiaagdirr rlseqlahtp tafkrdpedp
 541 savalkepwq ekvrriregs pyghlpnwrl lsvivkcgdd lrqellafqv lkqlqsiweq
 601 ervplwikpy kilvisadsg miepvvnavs ihqvkkqsql slldyflqeh gsytteafls
 661 aqrnfvqsca gyclvcyllq vkdrhngnil ldaeghiihi dfgfilsssp rnlgfetsaf
 721 klttefvdvm ggldgdmfny ykmlmlqgli aarkhmdkvv qiveimqqgs qlpcfhgsst
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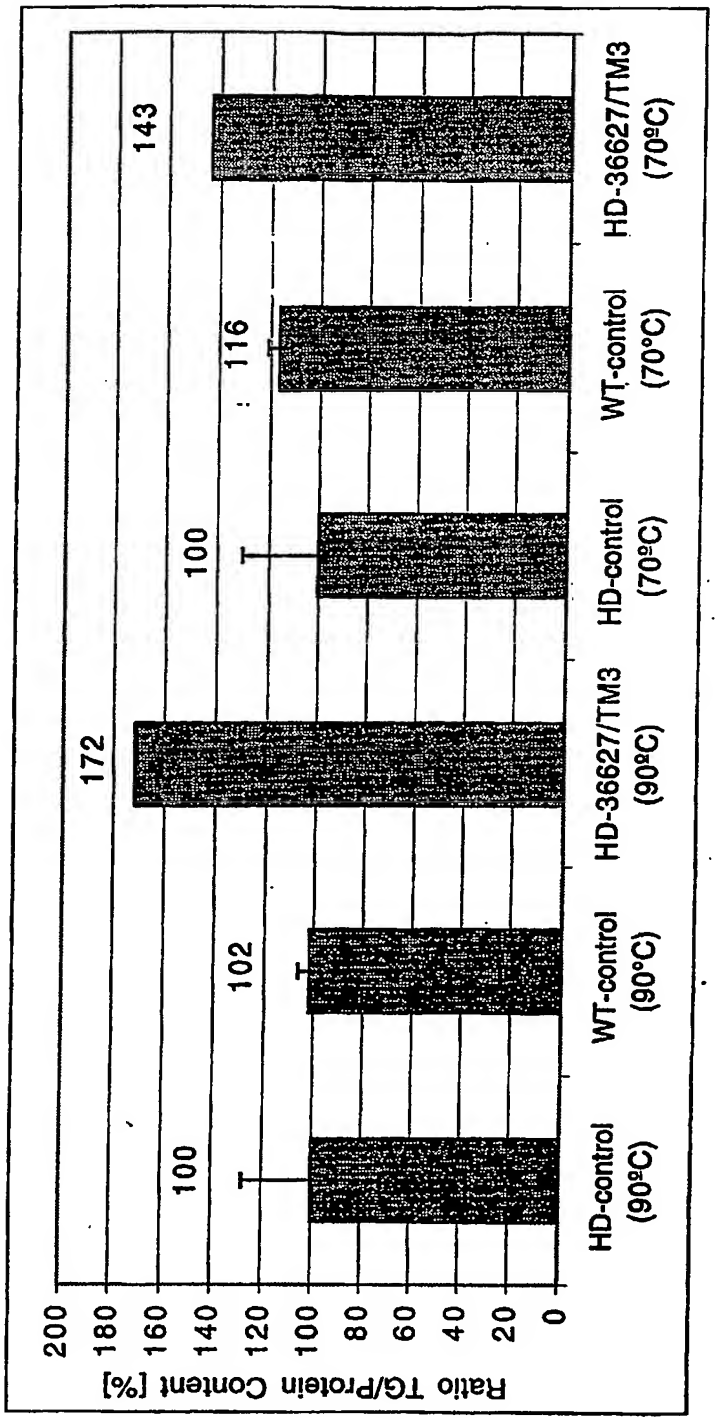

FIGURE 4. Energy storage metabolite content of a Drosophila Adk3 mutant (Gadfly Acc. No. CG6612)

Figure 5. Molecular organization of the Adk3 gene (GadFly Acession Number CG6612)

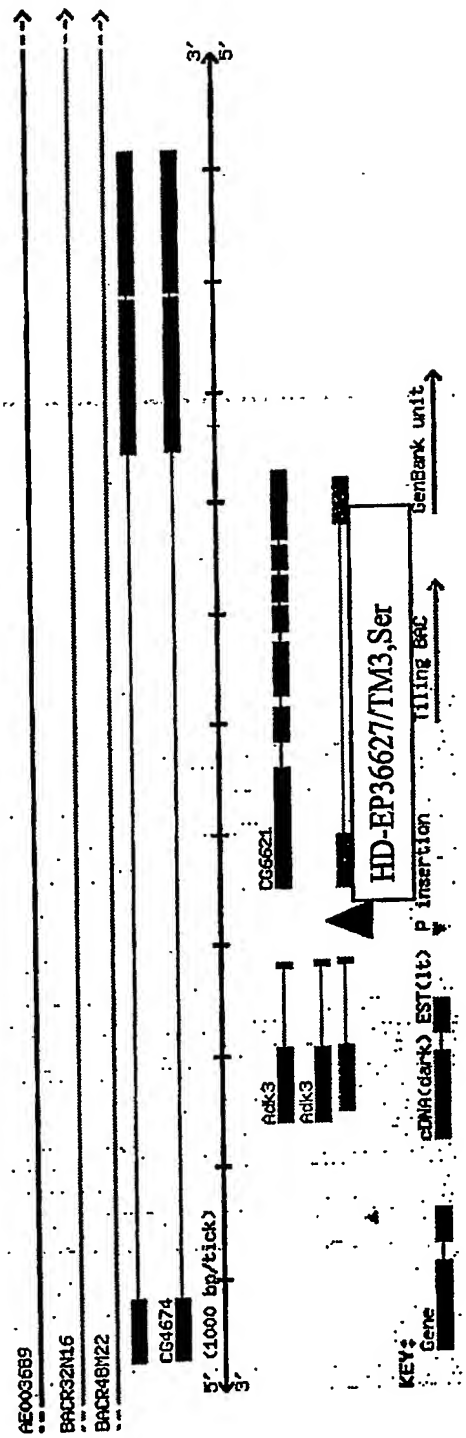

FIGURE 6. Nucleic acid sequences and amino acid sequences of the human adenylate kinase 3 and adenylate kinase 3 alpha like

FIGURE 6A. Homo sapiens adenylate kinase 3 (AK3), Nucleic acid sequence (SEQ ID NO: 3)

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   1 cggcgctggg ctgaggggag gggttgtctt aaaagtctct ccttcccccc gtaggggcgg
  61 ccggcgagtc ccagtgagag cggagggtgc cagaggtagg gggccgagaa acaaagttcc
 121 cggggcttcc tccggggccg cggtcggggc tgcgcgtttg accgcccccc tcctcgcgaa
 181 gcaatggctt ccaaactcct gcgcgcggtc atcctcgggc cgcccggctc gggcaagggc
 241 accgtgtgcc agaggatcgc ccagaacttt ggtctccagc atctctccag cggccacttc
 301 ttgcgggaga acatcaaggc cagcaccgaa gttggtgaga tggcaaagca gtatatagag
 361 aaaagtcttt tggttccaga ccatgtgatc acacgcctaa tgatgtccga gttggagaac
 421 aggcgtggac agcactggct ccttgatggt tttcctagga cattaggaca agccgaagcc
 481 ctggacaaaa tctgtgaagt ggatctagtg atcagtttga atattccatt tgaaacactt
 541 aaagatcgtc tcagccgccg ttggattcac cctcctagcg gaagggtata taacctggac
 601 ttcaatccac ctcatgtaca tggtattgat gacgtcactg gtgaaccgtt agtccagcag
 661 gaggatgata aacccgaagc agttgctgcc aggctaagac agtacaaaga cgtggcaaag
 721 ccagtcattg aattatacaa gagccgagga gtgctccacc aattttccgg aacggagacg
 781 aacaaaatct ggccctacgt ttacacactt ttctcaaaca agatcacacc tattcagtcc
 841 aaagaagcat attgaccctg cccaatggaa gaaccaggaa gatgtggtca ttcattcaat
 901 agtgtgtgta gtattggtgc tgtgtccaaa ttagaagcta gctgaggtag cttgcagcat
 961 cttttctagt tgaaatggtg aactgatagg aaaacaaatg agtagaaaga gttcatgaag
1021 aggccctcct ctgcctttca aaaggctggt cacctacaca tgtttaaggt gtctctgcac
1081 atgtctcaag cccatcacaa gaaagcaagt acagtgtgga tttcaaatgg tgtgtaactt
1141 cagctccagc tggtttttga cagctgttgc tgtggtaata tttttgacat gtgatggtga
1201 tagtctctgg ttctccccat ccccacaaag gctgttgaac cacagcacca ggaagcctga
1261 gaatgaatcc tgagggctct agcccaggct ttgtcccagg ctttctggtg tgtgccctcc
1321 tggtaacagt gaaattgaag ctacttactc atagtggttg tttctctggt cttgagtgac
1381 tgtgtccaca gttcattttt ttccggtagg aataactcct tttctacatc cacgctccat
1441 agagtctctc cttttcagac atcctgggat gaaagaattt ggctttttt tttctttttt
1501 ttttggacat ctgttttcac tcttaggctt ttaaacaata gttattgctt ttatccctct
1561 cagattctaa taactgagag cgatggggct atattgaatc tctgtatgca ctgagaactg
1621 agctatgaag agaatcttat taaactgctg gtctgacttt atggattgac actgttcctt
1681 tcttttattg tgaaaaaaaa aaaaaaa
```

FIGURE 6B. Homo sapiens adenylate kinase 3 (AK3), Amino acid sequence (SEQ ID NO: 4)

```
   1 maskllravi lgppgsgkgt vcqriaqnfg lqhlssghfl renikastev gemakqyiek
  61 sllvpdhvit rlmmselenr rgqhwlldgf prtlgqaeal dkicevdlvi slnipfetlk
 121 drlsrrwihp psgrvynldf npphvhgidd vtgeplvqqe ddkpeavaar lrqykdvakp
 181 vielyksrgv lhqfsgtetn kiwpyvytlf snkitpiqsk eay
```

FIGURE 6C. Homo sapiens adenylate kinase 3 alpha like (AKL3L), Nucleic acid sequence, isoform 1 (SEQ ID NO: 5)

```
   1 acttccggga acgccgggga accgcagtag ccgcctgcta gtggcgctgc tagccggccg
  61 gcgcaggctg ccgagcgggt gagcgcgcag gccaggccaa agccctggta cccgcgcggt
 121 gcgggcctca gtctgcggcc atgggggcgt ccgcgcggct gctgcgagcg gtgatcatgg
 181 gggccccggg ctcgggcaag ggcaccgtgt cgtcgcgcat cactacacac ttcgagctga
 241 agcacctctc cagcggggac ctgctccggg acaacatgct gcggggcaca gaaattggcg
 301 tgttagccaa ggctttcatt gaccaaggga aactcatccc agatgatgtc atgactcggc
```

```
 361 tggcccttca tgagctgaaa aatctcaccc agtatagctg gctgttggat ggttttccaa
 421 ggacacttcc acaggcagaa gccctagata gagcttatca gatcgacaca gtgattaacc
 481 tgaatgtgcc ctttgaggtc attaaacaac gccttactgc tcgctggatt catcccgcca
 541 gtggccgagt ctataacatt gaattcaacc ctcccaaaac tgtgggcatt gatgacctga
 601 ctggggagcc tctcattcag cgtgaggatg ataaaccaga gacggttatc aagagactaa
 661 aggcttatga agaccaaaca aagccagtcc tggaatatta ccagaaaaaa ggggtgctgg
 721 aaacattctc cggaacagaa accaacaaga tttggcccta tgtatatgct ttcctacaaa
 781 ctaaagttcc acaaagaagc cagaaagctt cagttactcc atgaggagaa atgtgtgtaa
 841 ctattaatag taagatgggc aaacctccta gtccttgcat ttagaagctg cttttcctaa
 901 gacttctagt atgtatgaat tctttgaaaa ttatattact tttatttcta ctgattttat
 961 tttggatact aaggatgtgc caaatgattc ggatactaag atgcatcgtt tgaaatcatc
1021 tagtgtgttg tatgcagtta tcctcaaaaa catcagcgat gtctgaacct ttaaaacatc
1081 tgttagagca aaattaaaag agcatttggt agtaatctaa ctttttgttc agttaataag
1141 tggttgataa agtttccata ttttttctgga aaagttaaaa aaagttacat gtcatttgga
1201 gaaaatacgt aatcagaaat ttgtgcatag attgatgcca aaaaagacat ttccagcatt
1261 gtggaacatg gtgagacact atataaaatt ccagaaagaa agcaactgga tttacagatt
1321 tattgtgaga cacaaattca ctgctgcctt tacactaaga aatgtatatg ttaaccatat
1381 atgctgtatt tattttgtcg ttaagcatac tttcagttta ctcagaattt tcaatttgct
1441 ataaagatgt atcaattagc atatagaaaa atattacttt aagatgactt gtttcctttg
1501 aaaatacctg tgtactgagg gttatgattt gtgtcaaaaa ttgacataag tgcttttaca
1561 agcaccaaag ttgaatgaat tttcaacaaa atgtaattaa agtctatgtt ttcagttatg
1621 actcaggtta agaaatgtgt tttaggatct acttgctggt ttttcttttt gatccaaatg
1681 tgtgatctgc cctgataaat aacaagttat agtaccatct cccccgccaa taaaaaagag
1741 aagaaaaaag agaaacccgt ggcactatgt aaataaagta agcatacttt gttgttagta
1801 aatagatgag gcatgcctgg gaaatgctcc cttggcataa atagcaatca attataatta
1861 gtaaacaggt gtaccaataa aaagaattta catgataggt taacaaggac caggaaagtg
1921 agtttcctga aggagttctt tgttcctgat caaagaaatt gatacctgtt agcattcact
1981 gccaccatat tttaaggaga aagaactcta ttggtgtcgt ctgagcagcc atttaaaaat
2041 tggaatctaa aggatggttg ctgatgtact gtgtggtctg gtagaagtgg ggaaatatga
2101 gagatggagg aaaaacttga ttatgtcttc catggcatat ttactcttac tttacttcgt
2161 gccaaatcaa atgaaacaag ccgtcttaca agtcgttatt gcctttaaaa atctgttccg
2221 ttttttcccc aggtacttaa aatacaagtg ccagtaagtg gttcttatgt gttttggggg
2281 gaaaatttta tttccctttt cttctgatat ttaaaaaatt catcgatctt tcaagatgaa
2341 ccaaggtttt ttaaaagaaa tataggaaac acttcattct ttataaaact ttctataatg
2401 ccttatttga atgttaatct tatgtgcttt ctaaaaaatg ttgtgaaata ccaaacttat
2461 ggattatcac taggttatca agcatatatt agtctttatc agaataaaat gaaatttcat
2521 aactgtggct attactttgt tcttggtcct tcacagggcc tgctccatcc caccttcctt
2581 tctgctgcct gatgtctcaa tggcttctga atgactgttc taataaatga tcttaaaaca
2641 gt
```

FIGURE 6D. Homo sapiens adenylate kinase 3 alpha like (AKL3L), Amino acid sequence, isoform 1 (SEQ ID NO: 6)

```
  1 mgasarllra vimgapgsgk gtvssritth felkhlssgd llrdnmlrgt eigvlakafi
 61 dqgklipddv mtrlalhelk nltqyswlld gfprtlpqae aldrayqidt vinlnvpfev
121 ikqrltarwi hpasgrvyni efnppktvgi ddltgepliq reddkpetvi krlkayedqt
181 kpvleyyqkk gvletfsgte tnkiwpyvya flqtkvpqrs qkasvtp
```

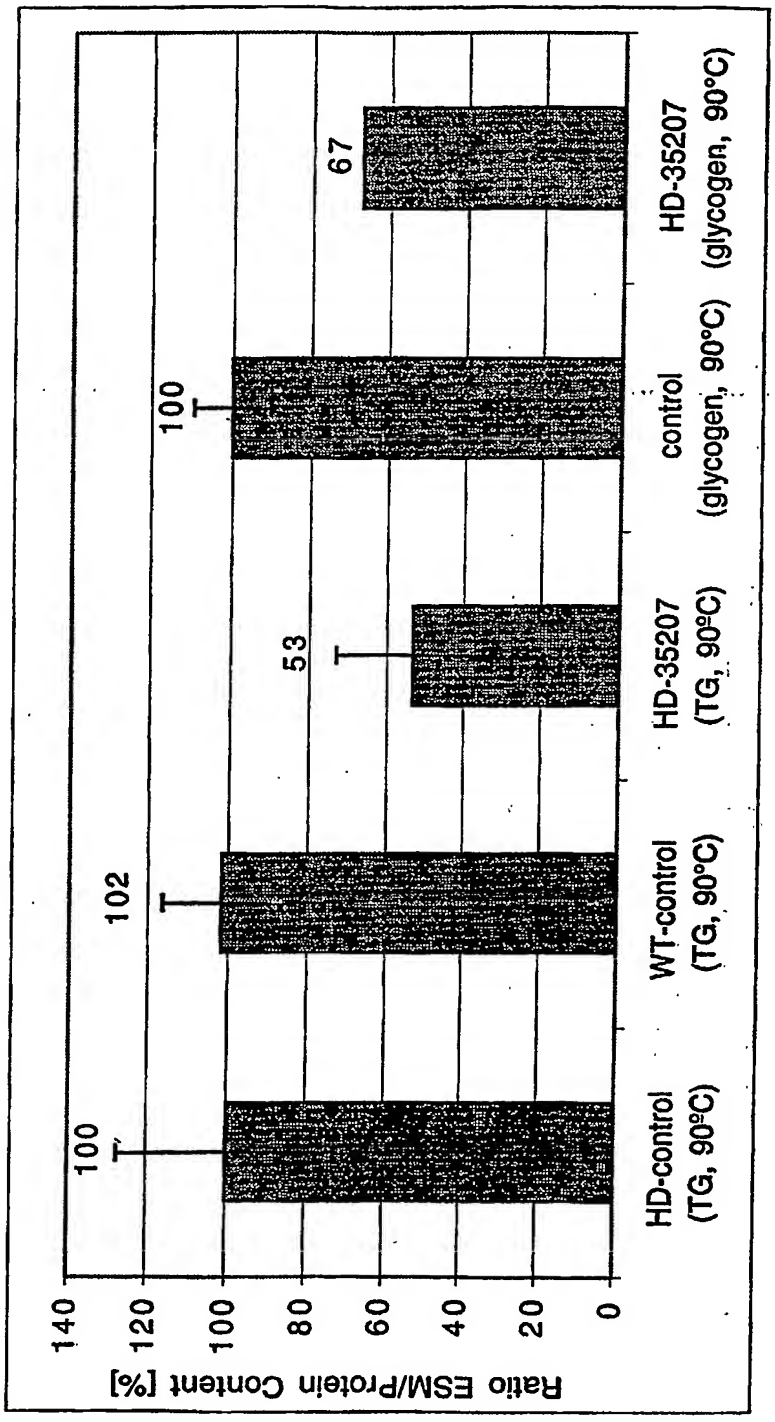

FIGURE 7. Energy storage metabolite content of a Drosophila Gdh (Gadfly Acc. No. CG5320) mutant

Figure 8. Molecular organization of the Gdh gene (GadFly Acession Number CG5320)

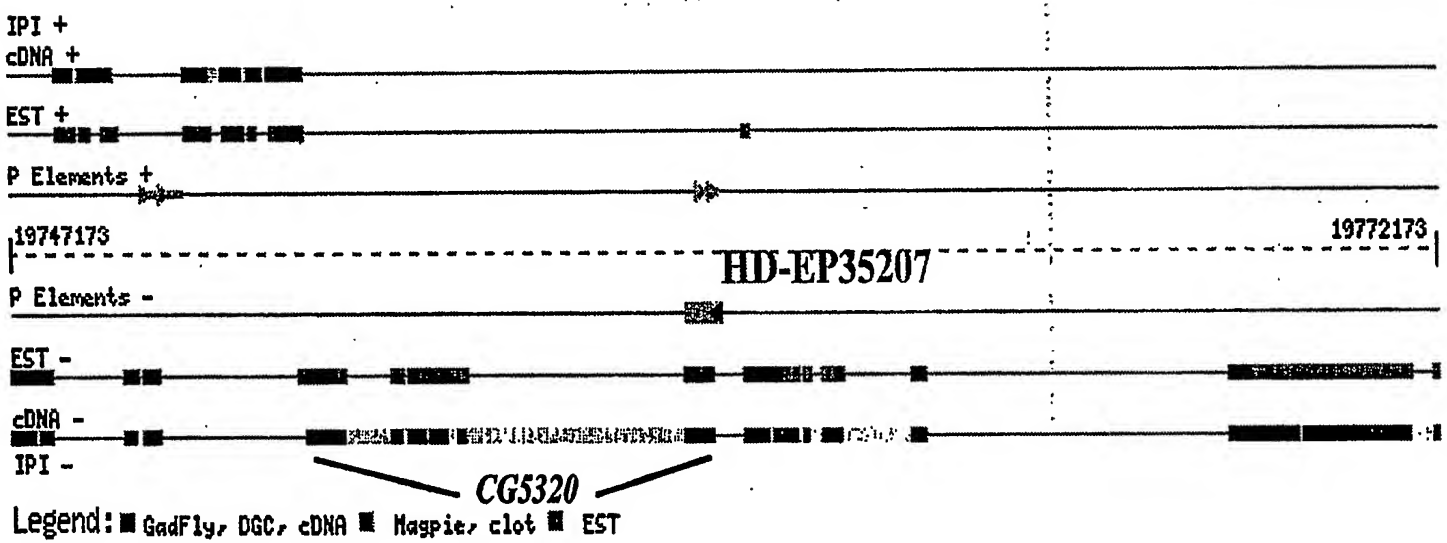

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FIGURE 9. Nucleic acid sequences and amino acid sequences of the human glutamate dehydrogenase 1 and glutamate dehydrogenase 2

FIGURE 9A. Homo sapiens glutamate dehydrogenase 1 (GLUD1), Nucleic acid sequence (SEQ ID NO: 7)

```
   1 gggcaacccg cgcgggaccc ttcctcccta gtcgcgggga gtctgagaaa gcgcgcctgt
  61 ttcgcgacca tcacgcacct cccctccgct tgtggccatg taccgctacc tgggcgaagc
 121 gctgttgctg tcccgggccg ggcccgctgc cctgggctcg gcgtccgccg actcggccgc
 181 gttgctgggc tgggcccggg gacagcccgc cgccgccccg cagccggggc tggcattggc
 241 cgcccggcgc cactacagcg aggcggtggc cgaccgcgag gacgacccca acttcttcaa
 301 gatggtggag ggcttcttcg atcgcggcgc cagcatcgtg gaggacaagc tggtggagga
 361 cctgaggacc cgggagagcg aggagcagaa gcggaaccgg gtgcgcggca tcctgcggat
 421 catcaagccc tgcaaccatg tgctgagtct ctccttcccc atccggcgcg acgacggctc
 481 ctgggaggtc atcgaaggct accgggccca gcacagccag caccgcacgc cctgcaaggg
 541 aggtatccgt tacagcactg atgtgagtgt agatgaagta aaagctttgg cttctctgat
 601 gacatacaag tgtgcagtgg ttgatgtgcc gtttgggggt gctaaagctg gtgttaagat
 661 caatcccaag aactatactg ataatgaatt ggaaaagatc acaaggaggt tcaccatgga
 721 gctagcaaaa aagggcttta ttggtcctgg cattgatgtg cctgctccag acatgagcac
 781 aggtgagcgg gagatgtcct ggatcgctga tacctatgcc agcaccatag ggcactatga
 841 tattaatgca cacgcctgtg ttactggtaa acccatcagc caaggggaa tccatggacg
 901 catctctgct actggccgtg gtgtcttcca tgggattgaa aatttcatca atgaagcttc
 961 ttacatgagc attttaggaa tgacaccagg gtttggagat aaaacatttg ttgttcaggg
1021 atttggtaat gtgggcctac actctatgag atatttacat cgttttggtg ctaaatgtat
1081 tgctgttggt gagtctgatg ggagtatatg gaatccagat ggtattgacc caaaggaact
1141 ggaagacttc aaattgcaac atgggtccat tctgggcttc cccaaggcaa agccctatga
1201 aggaagcatc ttggaggccg actgtgacat actgatccca gctgccagtg agaagcagtt
1261 gaccaaatcc aacgcaccca gagtcaaagc caagatcatt gctgaaggtg ccaatgggcc
1321 aacaactcca gaagctgaca agatcttcct ggagagaaac attatggtta ttccagatct
1381 ctacttgaat gctggaggag tgacagtatc ttactttgag tggctgaaga atctaaatca
1441 tgtcagctat ggccgtttga ccttcaaata tgaaagggat tctaactacc acttgctcat
1501 gtctgttcaa gagagtttag aaagaaaatt tggaaagcat ggtggaacta ttcccattgt
1561 acccacggca gagttccaag acaggatatc gggtgcatct gagaaagaca tcgtgcactc
1621 tggcttggca tacacaatgg agcgttctgc caggcaaatt atgcgcacag ccatgaagta
1681 taacctggga ttggacctga gaacagctgc ctatgttaat gccattgaga aagtcttcaa
1741 agtgtacaat gaagctggtg tgaccttcac atagatggat catggctgac ttcctcacta
1801 tcctcttcac atgtaacttc tgcagaccta tcacaagttt acatgtaacc acagaaatcc
1861 ctttctctcc tgactcatta ataatggata ccattctcaa caagtcaatc caagtcagcc
1921 cgttaaggag aaagaaatta aggttagcgg atcatgtaca agctgagtgt gaaagtagaa
1981 atcacctaca ccagagagcc attttggtat tttgccttta aataaaaagc ctcctttatc
2041 tggctgtgca gccttgctct gtggcttttc ccaacacaat cagtgctagt gctggggagg
2101 aacagtcaag agcagtcagt tgcttgctta ttttttctgg atgagtctgg gacacactgt
2161 aactttaaca catttaagaa gtaggtgtgt ggccttttca gaaggtggca tggtcctcaa
2221 gtgagttctt agtattttat atcagcaaaa taattcaatt ttgcaggttg caaacaaata
2281 taaaacctgt ttctgtttat gaatattatt cttttagaat agaataagta catgctgctg
2341 taataaaatt gcctttaatc acttaacaag cctaaccttg actcaaacag tgaatgccta
2401 tagaaataat aaatgaaaaa aactagtatt tttatatcat aaaacaatgt catttatagc
2461 ttatcattca tgtattgtcc agcagacatt aaaagccctg tggataatta agttatcttc
2521 atacctgcaa aatggtggag gctattttca ttaaaactgt cagaatttgc ttaccataat
2581 tatgatacag tccaaagaat gcagtcactt tttatcatgt taactaattg ttctcttttg
2641 aagatctatg gttgactaat taaacaataa ttcaagtaga gtgtcccaga aaaaaaccac
2701 ttgggctccc tgtttggagt ctggctggct ctgagcattg ccaatggccc ctactcacct
2761 gactttgtat cctctccttt tagaggcttt gcattctgca cccagcttca ctaacagtgg
2821 gctgaaaaca tccttgggtt gagtgtttca tttgggagtt atttggccag ggccttttga
2881 acagtagtgt ccccatgaag tgctagataa tatatgtgta agagtcagct ttttttttt
2941 tttttaactc taacaccctt cagaaatttc taactacttt gtaactgcat ggcttaacct
3001 ggtgataaaa gcagttatta aaagtctacg ttttccaaaa aaaaaaaaaa a
```

FIGURE 9B. Homo sapiens glutamate dehydrogenase 1, Amino acid sequence (SEQ ID NO: 8)

```
  1 myrylgeall lsragpaalg sasadsaall gwargqpaaa pqpglalaar rhyseavadr
 61 eddpnffkmv egffdrgasi vedklvedlr treseeqkrn rvrgilriik pcnhvlslsf
121 pirrddgswe viegyraqhs qhrtpckggi rystdvsvde vkalaslmty kcavvdvpfg
181 gakagvkinp knytdnelek itrrftmela kkgfigpgid vpapdmstge remswiadty
241 astighydin ahacvtgkpi sqggihgris atgrgvfhgi enfineasym silgmtpgfg
301 dktfvvqgfg nvglhsmryl hrfgakciav gesdgsiwnp dgidpkeled fklqhgsilg
361 fpkakpyegs ileadcdili paasekqltk snaprvkaki iaegangptt peadkifler
421 nimvipdlyl naggvtvsyf ewlknlnhvs ygrltfkyer dsnyhllmsv qeslerkfgk
481 hggtipivpt aefqdrisga sekdivhsgl aytmersarq imrtamkynl gldlrtaayv
541 naiekvfkvy neagvtft
```

FIGURE 9C. Homo sapiens Glutamate dehydrogenase-2 (GLUD2), Nucleic acid sequence (SEQ ID NO: 9)

```
   1 atgtaccgct acctggccaa agcgctgctg ccgtcccggg ccgggcccgc tgccctgggc
  61 tccgcggcca accactcggc cgcgttgctg ggccggggcc gcggacagcc cgccgccgcc
 121 tcgcagccgg ggctcgcatt ggccgcccgg cgccactaca gcgagttggt ggccgaccgc
 181 gaggacgacc ccaacttctt caagatggtg gagggcttct tcgatcgcgg cgccagcatc
 241 gtggaggaca agttggtgaa ggacctgagg acccaggaaa gcgaggagca gaagcggaac
 301 cgggtgcgcg gcatcctgcg gatcatcaag ccctgcaacc atgtgctgag tctctccttc
 361 cccatccggc gcgacgacgg ctcctgggag gtcatcgaag gctaccgggc ccagcacagc
 421 cagcaccgca cgccctgcaa gggaggtatc cgttacagca ctgatgtgag tgtagatgaa
 481 gtaaaagctt tggcttctct gatgacatac aagtgtgcag tggttgatgt gccgtttggg
 541 ggtgctaaag ctggtgttaa gatcaatccc aagaactata ccgaaaatga attggaaaag
 601 atcacaagga ggttcaccat ggagctagca aagaagggct ttattggtcc tggcgttgat
 661 gtgcctgctc cagacatgaa cacaggtgag cgggagatgt cctggattgc tgatacctat
 721 gccagcacca tagggcacta tgatattaat gcacacgcct gtgttactgg taaacccatc
 781 agccaagggg gaatccatgg acgcatctct gctactggcc gtggtgtctt ccatgggatt
 841 gaaaacttca tcaatcaagc ttcttacatg agcattttag gaatgacacc agggtttaga
 901 gataaaacat ttgttgttca gggatttggt aatgtgggcc tacactctat gagatattta
 961 catcgttttg gtgctaaatg tattgctgtt ggtgagtctg atgggagtat atggaatcca
1021 gatggtattg acccaaagga actggaagac ttcaaattgc aacatgggtc cattctgggc
1081 ttccccaagg caaagcccta tgaaggaagc atcttggagg tcgactgtga catactgatc
1141 ccagctgcca ctgagaagca gttgaccaaa tccaacgcac ccagagtcaa agccaagatc
1201 attgctgaag gtgccaatgg gccaacaact ccagaagctg ataagatctt cctggagaga
1261 aacattttgg ttattccaga tctctacttg aatgctggag gagtgacagt atcttacttt
1321 gagtggctga agaatctaaa tcatgtcagc tatggccgtt tgaccttcaa atatgaaagg
1381 gattctaact accacttgct cctgtctgtt caagagagtt tagaaagaaa atttggaaag
1441 catggtggaa ctattcccat tgtacccacg gcagagttcc aagacagtat atcgggtgca
1501 tctgagaaag acatcgtgca ctctgccttg gcatacacaa tggagcgttc tgccaggcaa
1561 attatgcaca cagccatgaa gtataacctg ggattggacc tgagaacagc tgcctatgtc
1621 aatgccattg aaaaagtctt caaagtgtac agtgaagctg gtgtgacctt cacatag
```

FIGURE 9D. Homo sapiens Glutamate dehydrogenase-2, Amino acid sequence (SEQ ID NO: 10)

```
  1 myrylakall psragpaalg saanhsaall grgrgqpaaa sqpglalaar rhyselvadr
 61 eddpnffkmv egffdrgasi vedklvkdlr tqeseeqkrn rvrgilriik pcnhvlslsf
```

```
121 pirrddgswe viegyraqhs qhrtpckggi rystdvsvde vkalaslmty kcavvdvpfg
181 gakagvkinp knytenelek itrrftmela kkgfigpgvd vpapdmntge remswiadty
241 astighydin ahacvtgkpi sqggihgris atgrgvfhgi enfinqasym silgmtpgfr
301 dktfvvqgfg nvglhsmryl hrfgakciav gesdgsiwnp dgidpkeled fklqhgsilg
361 fpkakpyegs ilevdcdili paatekqltk snaprvkaki iaegangptt peadkifler
421 nilvipdlyl naggvtvsyf ewlknlnhvs ygrltfkyer dsnyhlllsv qeslerkfgk
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541 naiekvfkvy seagvtft
```

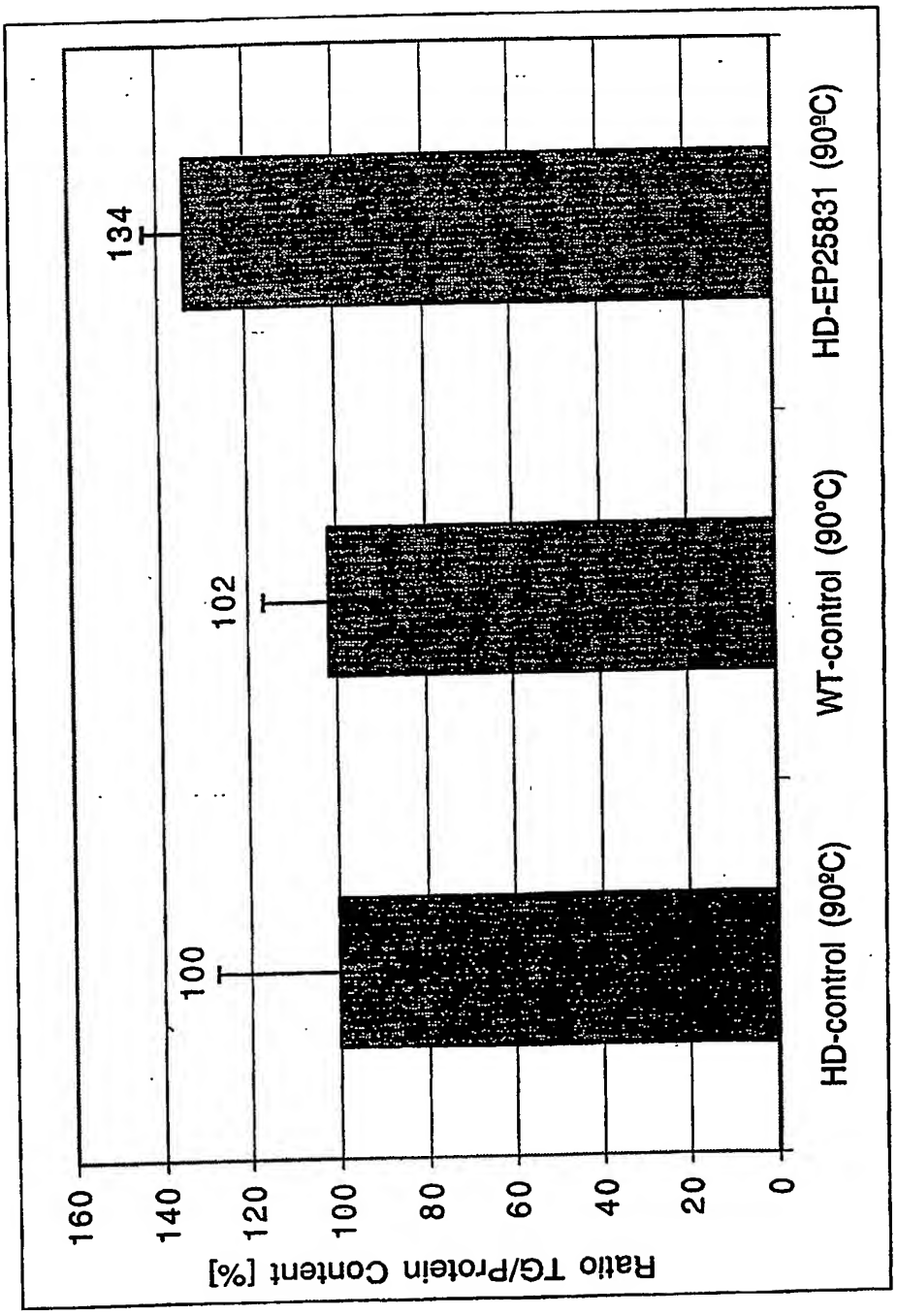

FIGURE 10. Energy storage metabolite content of a Drosophila CG3860 (Gadfly Acc. No.) mutant

FIGURE 11. Molecular organization of the CG3860 gene (GadFly Acession Number)

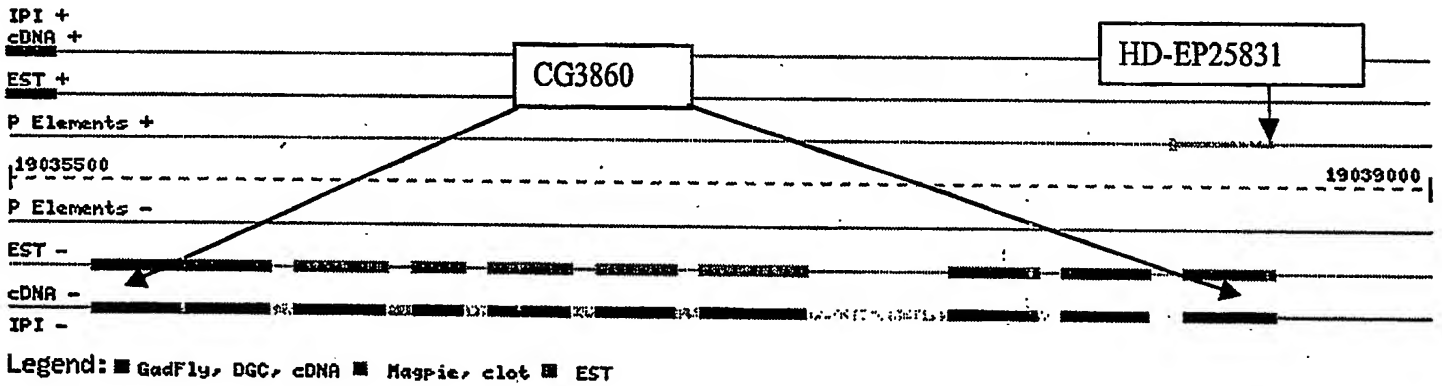

FIGURE 12. Nucleic acid sequences and amino acid sequences of the human Homo sapiens oxysterol binding protein-like 1A and 2

FIGURE 12A. Homo sapiens oxysterol binding protein-like 1A (OSBPL1A), Nucleic acid sequence, transcript variant OSBPL1A (SEQ ID NO: 11)

```
   1 ccagggccgg tgtctccagg agagggccga gcgcacagca gctctaggac ctcgcaggcg
  61 cctcggcgcg gcactcacgt agagcccaag gagcagtccc cgacctctcc tcggagggcc
 121 gtgcaggctt ccccggcggt ccctacctgc gcctccgccc gccagcggcg ctggggaagg
 181 ggaggagcgg cccggacgct gcccgccgcg cgcagggagc cgcccgccgc aggccttgcc
 241 gccgcccgcc cgcgctctcc ccgcgagccc cggagggcgg gtctgcggcc gagccagcgg
 301 cggcggaggg gcagcgcaga cctctgcggc ccgggcgggc gcggcgcggg agtcctgggc
 361 cgctggcggg caacgcctct gcccgacctc gctggggaag gctggcgctg ctgcccggcc
 421 ggagccaggg gcaggctgcg caaaggtgac ttagggagac cttgcgggcc gccctgggtc
 481 gcgcctcccg gatcggctcg cctcggctcg actggagggg aggaggagga gcaggccgag
 541 cgcattcgcg ctggagcttg cgaggagcgc agggtggagc gcgccagccg gggtcctcgg
 601 atctggccca ggtgaggaat tttaaattgg aacaagagca agaaaaaaac aaaatcttgt
 661 cagaagcact ggagacgctg gccactgaac atcatgaatt agagcagtct ctggtgaaag
 721 gctctccacc cgccagcatc cttagcgagg acgagttcta tgatgcgctg tcagattccg
 781 agtccgaaag gtccctgagt agattggaag cagtgacagc acgctccttt gaagaggaag
 841 gagagcattt gggcagtaga aaacacagaa tgtccgaaga aaaagactgt ggtggcggag
 901 atgctctctc caatggcatc aagaaacaca gaacaagttt gccttctcct atgttttcca
 961 gaaatgactt cagtatctgg agcatcctca gaaaatgtat tggaatggaa ctatccaaga
1021 tcacgatgcc agttatattt aatgagcctc tgagcttcct acagcgccta actgaataca
1081 tggagcatac ttacctcatc cacaaggcca gttcactctc tgatcctgtg gaaaggatgc
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1201 ctttcaaccc actgctggga gagacttatg aattagtgcg agatgacctt ggatttagac
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1321 acaatgactt catctttcat ggctctatct atcccaaact gaaattctgg gggaagagtg
1381 tagaagcaga acccaaagga accatcacct tggagctcct tgaacacaat gaggcatata
1441 catggacaaa tcccacctgc tgtgtgcata atatcattgt gggtaaactg tggatcgaac
1501 agtatggcaa tgtggaaatt ataaaccaca agactgggga caaatgtgtg ttgaatttta
1561 agccatgtgg ccttttggt aaggaattac acaaagttga aggctacatt caagataaaa
1621 gcaaaaagaa gctctgtgcc ctctatggga agtggactga atgtttatac agtgttgacc
1681 ctgccacgtt tgacgcttac aaaaaaaatg ataagaaaaa tacagaagag aagaagaaca
1741 gcaaacagat gagcacctct gaggagttgg atgaaatgcc agtgccggat tctgaaagtg
1801 tattcattat ccctggaagc gttcttctat ggcgaatagc cccacggcct ccaaattctg
1861 cccagatgta taattttact agttttgcaa tggttttgaa tgaagtagac aaagacatgg
1921 agagtgtgat tcccaagaca gactgcaggt tacggcctga catcagagcc atggaaaatg
1981 gagagataga tcaagctagt gaagaaaaaa aacgacttga ggaaaaacaa agagcagccc
2041 gcaaaaacag gtccaagtca gaagaggact ggaagacgag gtggttccat caaggtccta
2101 atccctacaa tggagcacag gactggattt actctggcag ctactgggac agaaattact
2161 tcaatttgcc tgacatttat taaaatgcat acaagtcagg gtgtttggct aatctacaaa
2221 taagtcttaa acctatgttt ttaaattttt ttcccttggt ttctacttat cttttaaaaa
2281 aaaaatgaaa aaacactcat gagataactg catttcaccc aacaaaagca gggtataagg
2341 cgatattggt gatgaaagtc ttaggaaaaa tgcataattt tgctataaaa tgtacttatt
2401 tggaatacta ttttatatag aggtaagaga acactgctgg ggaatatgct ttttatggtt
2461 gctgttgcca tatttactga aggtttatac ctaaatgtaa ctttagcttt atggaactat
2521 atagtaatcc caaatcaagt tattttgaat atttttatgc tgtcatgctt gaatgtttta
2581 gatgtaacct ttgacatatt tagaactctc ctcctataca atgtttattc tcagatatag
2641 aggttatgtc attttataaa gacttcattg ataagatggc ttttattcat actaatcctc
2701 ccaatgttac cccttccatc ttccaagaag aaaaaaaatg cctgaatatt cagaatagat
2761 atttctgatt tgaaaattct aaagaattaa actggaaaag tatttcattt acttagtgct
2821 ctgaatttac ttttacagtt ttctgcagtc agtatcatta aaatggttaa gtttacattt
2881 gaactgaaaa tatgtataaa atctagcaat tcacaaaaat gccctagaaa tatagatttt
2941 aatcaccatt acataatgac aaaccttgtt aaatgcttcc acttccagtg gcaaatgcca
3001 ctagggaaag taagttgcac tcatgtaagt atcaaactat ataaaaggag gccttgtgca
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3061 tttcaagttt gcaaagtacc tgtgtactta aaatatgtgt ggagacctac tgtacagtag
3121 ttttgcccct ttaattgggg cacattcatc ttaaatctta tagtatttat ccacccaaac
3181 cccagactga gatactgctc ccaggggcct aggtagctgc cagtccgtga ttttaattgc
3241 tgtcttgaag ttaacaagtg ttataatgaa ataatctacc tgatgctaaa taaaggcttt
3301 agaatgttcc ccaaaaaaaa aaaaaaaaaa aaaaaa
```

FIGURE 12B. Homo sapiens oxysterol-binding protein-like 1A (OSBPL1A), Amino acid sequence, isoform A (SEQ ID NO: 12)

```
   1 mseekdcggg dalsngikkh rtslpspmfs rndfsiwsil rkcigmelsk itmpvifnep
  61 lsflqrltey mehtylihka sslsdpverm qcvaafavsa vasqwertgk pfnpllgety
 121 elvrddlgfr liseqvshhp pisafhaegl nndfifhgsi ypklkfwgks veaepkgtit
 181 lellehneay twtnptccvh niivgklwie qygnveiinh ktgdkcvlnf kpcglfgkel
 241 hkvegyiqdk skkklcalyg kwteclysvd patfdaykkn dkknteekkn skqmstseel
 301 dempvpdses vfiipgsvll wriaprppns aqmynftsfa mvlnevdkdm esvipktdcr
 361 lrpdiramen geidqaseek krleekqraa rknrskseed wktrwfhqgp npyngaqdwi
 421 ysgsywdrny fnlpdiy
```

FIGURE 12C. Homo sapiens oxysterol binding protein-like 1A (OSBPL1A), Nucleic acid sequence, transcript variant OSBPL1B (SEQ ID NO: 13)

```
   1 ccgtggtccc ggcgccgggt cccggagaca gacgttacgc gggctcgagc gtcctcgggg
  61 agtgccagcc agagttggtg acgaccactt cctcgacgtg gggcgggcgg acgggaagcc
 121 tggggtcgtg gccaccgcct cgggagctct gggagcccgg gtgaccgcgt agaaatgaac
 181 acagaagcgg agcaacagct tctccatcac gccagaaatg gcaatgctga agaagtaaga
 241 caactattag agaccatggc gaggaatgaa gtgattgctg acattaattg caaaggaaga
 301 agtaagtcta acttgggctg gacacctcta catctggcat gctattttgg acacagacaa
 361 gtggtccagg atctgttgaa ggctggtgca gaagtgaatg tgttgaatga catgggagac
 421 acgccgcttc atcgagctgc ctttacagga cgaaaggagt tggtaatgct tctcttagaa
 481 tataatgctg atactactat tgttaatggg agtggacaga cagcaaaaga agttactcat
 541 gctgaagaaa tcagaagcat gcttgaagct gtagaaagga ctcaacaaag aaagcttgaa
 601 gaattacttt tagcagcagc aagagaaggc aaaacaacag aactcacagc tctgctcaac
 661 aggcccaatc ctcctgatgt taactgttcg gatcagttag gaaatacacc cttgcattgt
 721 gcagcttacc gggcccataa acaatgtgcc ttaaagcttc taagaagtgg agcagaccct
 781 aatctgaaga acaaaaatga tcagaaacct cttgaccttg cccagggtgc tgaaatgaaa
 841 cacattcttg ttggtaataa ggtcatctac aaagcattga aacgatatga gggccctctc
 901 tggaagagtt caagattttt tggctggaga ttattctggg tagtgttaga gcatggagtc
 961 ctttcatggt ataggaaaca gcctgatgca gttcataata tttatcgcca gggatgcaaa
1021 cacctgactc aagcagtatg cacggtaaaa tccactgata gctgcctctt ctttattaaa
1081 tgctttgatg acaccattca tggcttccgg gttcctaaga atagccttca gcagtcaaga
1141 gaggactggc tggaagcaat agaagaacat tctgcttaca gcactcacta ctgttcccag
1201 gaccagctga ctgatgagga ggaggaagat acggtttctg ctgcagacct gaagaaatca
1261 ttagagaaag cacagtcatg ccaacagcga ctagataggg aaatttccaa ctttctcaaa
1321 atgattaagg agtgtgacat ggctaaagaa atgcttccat catttcttca gaaagttgaa
1381 gttgtctcag aagcttctag agaaacttgt gtagctttga ctgattgcct taatctcttc
1441 accaaacaag aaggggtgag gaatttttaaa ttggaacaag agcaagaaaa aaacaaaatc
1501 ttgtcagaag cactggagac gctggccact gaacatcatg aattagagca gtctctggtg
1561 aaaggctctc cacccgccag catccttagc gaggacgagt tctatgatgc gctgtcagat
1621 tccgagtccg aaaggtccct gagtagattg gaagcagtga cagcacgctc ctttgaagag
1681 gaaggagagc atttgggcag tagaaaacac agaatgtccg aagaaaaaga ctgtggtggc
1741 ggagatgctc tctccaatgg catcaagaaa cacagaacaa gtttgccttc tcctatgttt
1801 tccagaaatg acttcagtat ctggagcatc ctcagaaaat gtattggaat ggaactatcc
1861 aagatcacga tgccagttat atttaatgag cctctgagct tcctacagcg cctaactgaa
1921 tacatggagc atacttacct catccacaag gccagttcac tctctgatcc tgtggaaagg
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1981 atgcagtgtg tagctgcgtt tgctgtatct gctgttgctt ctcagtggga acggactgga
2041 aaacctttca acccactgct gggagagact tatgaattag tgcgagatga ccttggattt
2101 agactcatct ccgaacaggt cagccatcac ccaccaatca gtgcatttca tgctgaagga
2161 ttaaacaatg acttcatctt tcatggctct atctatccca aactgaaatt ctgggggaag
2221 agtgtagaag cagaacccaa aggaaccatc accttggagc tccttgaaca caatgaggca
2281 tatacatgga caaatcccac ctgctgtgtg cataatatca ttgtgggtaa actgtggatc
2341 gaacagtatg gcaatgtgga aattataaac cacaagactg gggacaaatg tgtgttgaat
2401 tttaagccat gtggcctttt tggtaaggaa ttacacaaag ttgaaggcta cattcaagat
2461 aaaagcaaaa agaagctctg tgccctctat gggaagtgga ctgaatgttt atacagtgtt
2521 gaccctgcca cgtttgacgc ttacaaaaaa aatgataaga aaaatacaga agagaagaag
2581 aacagcaaac agatgagcac ctctgaggag ttggatgaaa tgccagtgcc ggattctgaa
2641 agtgtattca ttatccctgg aagcgttctt ctatggcgaa tagccccacg gcctccaaat
2701 tctgcccaga tgtataattt tactagtttt gcaatggttt tgaatgaagt agacaaagac
2761 atggagagtg tgattcccaa gacagactgc aggttacggc ctgacatcag agccatggaa
2821 aatggagaga tagatcaagc tagtgaagaa aaaaaacgac ttgaggaaaa acaaagagca
2881 gcccgcaaaa acaggtccaa gtcagaagag gactggaaga cgaggtggtt ccatcaaggt
2941 cctaatccct acaatggagc acaggactgg atttactctg gcagctactg ggacagaaat
3001 tacttcaatt tgcctgacat ttattaaaat gcatacaagt cagggtgttt ggctaatcta
3061 caaataagtc ttaaacctat gtttttaaat ttttttccct tggtttctac ttatctttta
3121 aaaaaaaaat gaaaaaacac tcatgagata actgcatttc acccaacaaa agcagggtat
3181 aaggcgatat tggtgatgaa agtcttagga aaaatgcata attttgctat aaaatgtact
3241 tatttggaat actatttttat atagaggtaa gagaacactg ctggggaata tgctttttat
3301 ggttgctgtt gccatattta ctgaaggttt atacctaaat gtaactttag ctttatggaa
3361 ctatatagta atcccaaatc aagttatttt gaatattttt atgctgtcat gcttgaatgt
3421 tttagatgta acctttgaca tatttagaac tctcctccta tacaatgttt attctcagat
3481 atagaggtta tgtcatttta taaagacttc attgataaga tggcttttat tcatactaat
3541 cctcccaatg ttaccccttc catcttccaa gaagaaaaaa aatgcctgaa tattcagaat
3601 agatatttct gatttgaaaa ttctaaagaa ttaaactgga aaagtatttc atttacttag
3661 tgctctgaat ttacttttac agttttctgc agtcagtatc attaaaatgg ttaagtttac
3721 atttgaactg aaaatatgta taaaatctag caattcacaa aaatgcccta gaaatataga
3781 ttttaatcac cattacataa tgacaaacct tgttaaatgc ttccacttcc agtggcaaat
3841 gccactaggg aaagtaagtt gcactcatgt aagtatcaaa ctatataaaa ggaggccttg
3901 tgcatttcaa gtttgcaaag tacctgtgta cttaaaatat gtgtggagac ctactgtaca
3961 gtagttttgc ccctttaatt ggggcacatt catcttaaat cttatagtat ttatccaccc
4021 aaaccccaga ctgagatact gctcccaggg gcctaggtag ctgccagtcc gtgattttaa
4081 ttgctgtctt gaagttaaca agtgttataa tgaaataatc tacctgatgc taaataaagg
4141 ctttagaatg ttccccaaaa aaaaa
```

FIGURE 12D. Homo sapiens oxysterol-binding protein-like 1A (OSBPL1A), Amino acid sequence, isoform B (SEQ ID NO: 14)

```
  1 mnteaeqqll hharngnaee vrqlletmar neviadinck grsksnlgwt plhlacyfgh
 61 rqvvqdllka gaevnvlndm gdtplhraaf tgrkelvmll leynadttiv ngsgqtakev
121 thaeeirsml eavertqqrk leelllaaar egktteltal lnrpnppdvn csdqlgntpl
181 hcaayrahkq calkllrsga dpnlknkndq kpldlaqgae mkhilvgnkv iykalkryeg
241 plwkssrffg wrlfwvvleh gvlswyrkqp davhniyrqg ckhltqavct vkstdsclff
301 ikcfddtihg frvpknslqq sredwleaie ehsaysthyc sqdqltdeee edtvsaadlk
361 kslekaqscq qrldreisnf lkmikecdma kemlpsflqk vevvseasre tcvaltdcln
421 lftkqegvrn fkleqeqekn kilsealetl atehheleqs lvkgsppasi lsedefydal
481 sdsesersls rleavtarsf eeegehlgsr khrmseekdc gggdalsngi kkhrtslpsp
541 mfsrndfsiw silrkcigme lskitmpvif neplsflqrl teymehtyli hkasslsdpv
601 ermqcvaafa vsavasqwer tgkpfnpllg etyelvrddl gfrliseqvs hhppisafha
661 eglnndfifh gsiypklkfw gksveaepkg titlellehn eaytwtnptc cvhniivgkl
721 wieqygnvei inhktgdkcv lnfkpcglfg kelhkvegyi qdkskkklca lygkwtecly
781 svdpatfday kkndkkntee kknskqmsts eeldempvpd sesvfiipgs vllwriaprp
841 pnsaqmynft sfamvlnevd kdmesvipkt dcrlrpdira mengeidqas eekkrleekq
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901 raarknrsks eedwktrwfh qgpnpyngaq dwiysgsywd rnyfnlpdiy

FIGURE 12E. Homo sapiens oxysterol binding protein-like 1A (OSBPL1A), Nucleic acid sequence, transcript variant OSBPL1C (SEQ ID NO: 15)

```
   1 ccgtggtccc ggcgccgggt cccggagaca gacgttacgc gggctcgagc gtcctcgggg
  61 agtgccagcc agagttggtg acgaccactt cctcgacgtg gggcgggcgg acgggaagcc
 121 tggggtcgtg gccaccgcct cgggagctct gggagcccgg gtgaccgcgt agaaatgaac
 181 acagaagcgg agcaacagct tctccatcac gccagaaatg gcaatgctga agaagtaaga
 241 caactattag agaccatggc gaggaatgaa gtgattgctg acattaattg caaaggaaga
 301 agtaagtcta acttgggctg gacacctcta catctggcat gctattttgg acacagacaa
 361 gtggtccagg atctgttgaa ggctggtgca gaagtgaatg tgttgaatga catgggagac
 421 acgccgcttc atcgagctgc ctttacagga cgaaaggagt tggtaatgct tctcttagaa
 481 tataatgctg atactactat tgttaatggg agtggacaga cagcaaaaga agttactcat
 541 gctgaagaaa tcagaagcat gcttgaagct gtagaaagga ctcaacaaag aaagcttgaa
 601 gaattacttt tagcagcagc aagagaaggc aaaacaacag aactcacagc tctgctcaac
 661 aggcccaatc ctcctgatgt taactgttcg gatcagttag gaaatacacc cttgcattgt
 721 gcagcttacc gggcccataa acaatgtgcc ttaaagcttc taagaagtgg agcagaccct
 781 aatctgaaga acaaaaatga tcagaaacct cttgaccttg cccagggtgc tgaaatgaaa
 841 cacattcttg ttggtaataa ggtcatctac aaagcattga aacgatatga gggccctctc
 901 tggaagagtt caagattttt tggctggaga ttattctggg tagtgttaga gcatggagtc
 961 ctttcatggt ataggaaaca gcctgatgca gttcataata tttatcgcca gggatgcaaa
1021 cacctgactc aagcagtatg cacggtaaaa tccactgata gctgcctctt ctttattaaa
1081 tgctttgatg acaccattca tggcttccgg gttcctaaga atagccttca gcagtcaaga
1141 gaggactggc tggaagcaat agaagaacat tctgcttaca gcactcacta ctgttcccag
1201 gaccagctga ctgatgagga ggaggaagat acggtttctg ctgcagacct gaagaaatca
1261 ttagagaaag cacagtcatg ccaacagcga ctagataggg aaatttccaa ctttctcaaa
1321 atgattaagg agtgtgacat ggctaaagaa atgcttccat catttcttca gaaagttgaa
1381 gttgtctcag aagcttctag agaaacttgt gtagctttga ctgattgcct taatctcttc
1441 accaaacaag aaggggtgag gaattttaaa ttggaacaag agcaagaaaa aaacaaaatc
1501 ttgtcagaag cactggagac gctggccact gaacatcatg aattagagca gtctctggtg
1561 aaaggctctc cacccgccag catccttagc gaggacgagt tctatgatgc gctgtcagat
1621 tccgagtccg aaaggtccct gagtagattg gaagcagtga cagcacgctc ctttgaagag
1681 gaaggagagc atttgggcag tagaaaacac agaatgtccg aagaaaaaga ctgtggtggc
1741 ggagatgctc tctccaatgg catcaagaaa cacagaacaa gtttgccttc tcctatgttt
1801 tccagaaatg acttcagtat ctggagcatc ctcagaaaat gtattggaat ggaactatcc
1861 aagatcacga tgccagttat atttaatgag cctctgagct tcctacagcg cctaactgaa
1921 tacatggagc atacttacct catccacaag gccagttcac tctctgatcc tgtggaaagg
1981 atgcagtgtg tagctgcgtt tgctgtatct gctgttgctt ctcagtggga acggactgga
2041 aaacctttgg tcagccatca cccaccaatc agtgcatttc atgctgaagg attaaacaat
2101 gacttcatct ttcatggctc tatctatccc aaactgaaat tctgggggaa gagtgtagaa
2161 gcagaaccca aaggaaccat caccttggag ctccttgaac acaatgaggc atatacatgg
2221 acaaatccca cctgctgtgt gcataatatc attgtgggta aactgtggat cgaacagtat
2281 ggcaatgtgg aaattataaa ccacaagact ggggacaaat gtgtgttgaa ttttaagcca
2341 tgtggccttt ttggtaagga attacacaaa gttgaaggct acattcaaga taaaagcaaa
2401 aagaagctct gtgccctcta tgggaagtgg actgaatgtt tatacagtgt tgaccctgcc
2461 acgtttgacg cttacaaaaa aaatgataag aaaaatacag aagagaagaa gaacagcaaa
2521 cagatgagca cctctgagga gttggatgaa atgccagtgc cggattctga aagtgtattc
2581 attatccctg gaagcgttct tctatggcga atagccccac ggcctccaaa ttctgcccag
2641 atgtataatt ttactagttt tgcaatggtt ttgaatgaag tagacaaaga catggagagt
2701 gtgattccca agacagactg caggttacgg cctgacatca gagccatgga aaatggagag
2761 atagatcaag ctagtgaaga aaaaaaacga cttgaggaaa aacaaagagc agcccgcaaa
2821 aacaggtcca agtcagaaga ggactggaag acgaggtggt tccatcaagg tcctaatccc
2881 tacaatggag cacaggactg gatttactct ggcagctact gggacagaaa ttacttcaat
2941 ttgcctgaca tttattaaaa tgcatacaag tcagggtgtt tggctaatct acaaataagt
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3001 cttaaaccta tgtttttaaa ttttttttccc ttggtttcta cttatctttt aaaaaaaaaa
3061 tgaaaaaaca ctcatgagat aactgcattt cacccaacaa aagcagggta taaggcgata
3121 ttggtgatga aagtcttagg aaaaatgcat aattttgcta taaaatgtac ttatttggaa
3181 tactatttta tatagaggta agagaacact gctggggaat atgcttttta tggttgctgt
3241 tgccatattt actgaaggtt tatacctaaa tgtaacttta gctttatgga actatatagt
3301 aatcccaaat caagttattt tgaatatttt tatgctgtca tgcttgaatg ttttagatgt
3361 aacctttgac atatttagaa ctctcctcct atacaatgtt tattctcaga tatagaggtt
3421 atgtcatttt ataaagactt cattgataag atggctttta ttcatactaa tcctcccaat
3481 gttacccctt ccatcttcca agaagaaaaa aaatgcctga atattcagaa tagatatttc
3541 tgatttgaaa attctaaaga attaaactgg aaaagtattt catttactta gtgctctgaa
3601 tttactttta cagttttctg cagtcagtat cattaaaatg gttaagttta catttgaact
3661 gaaaatatgt ataaaatcta gcaattcaca aaaatgccct agaaatatag attttaatca
3721 ccattacata atgacaaacc ttgttaaatg cttccacttc cagtggcaaa tgccactagg
3781 gaaagtaagt tgcactcatg taagtatcaa actatataaa aggaggcctt gtgcatttca
3841 agtttgcaaa gtacctgtgt acttaaaata tgtgtggaga cctactgtac agtagttttg
3901 ccccttaat tggggcacat tcatcttaaa tcttatagta tttatccacc caaaccccag
3961 actgagatac tgctcccagg ggcctaggta gctgccagtc cgtgatttta attgctgtct
4021 tgaagttaac aagtgttata atgaaataat ctacctgatg ctaaataaag gctttagaat
4081 gttccccaaa aaaaaa
```

FIGURE 12F. Homo sapiens oxysterol-binding protein-like 1A (OSBPL1A), Amino acid sequence, isoform C (SEQ ID NO: 16)

```
   1 mnteaeqql1 hharngnaee vrqlletmar neviadinck grsksnlgwt plhlacyfgh
  61 rqvvqdllka gaevnvlndm gdtplhraaf tgrkelvmll leynadttiv ngsgqtakev
 121 thaeeirsml eavertqqrk leelllaaar egktteltal lnrpnppdvn csdqlgntpl
 181 hcaayrahkq calkllrsga dpnlknkndq kpldlaqgae mkhilvgnkv iykalkryeg
 241 plwkssrffg wrlfwvvleh gvlswyrkqp davhniyrqg ckhltqavct vkstdsclff
 301 ikcfddtihg frvpknslqq sredwleaie ehsaysthyc sqdqltdeee edtvsaadlk
 361 kslekaqscq qrldreisnf lkmikecdma kemlpsflqk vevvseasre tcvaltdcln
 421 lftkqegvrn fkleqeqekn kilsealetl atehheleqs lvkgsppasi lsedefydal
 481 sdsesersls rleavtarsf eeegehlgsr khrmseekdc gggdalsngi kkhrtslpsp
 541 mfsrndfsiw silrkcigme lskitmpvif neplsflqrl teymehtyli hkasslsdpv
 601 ermqcvaafa vsavasqwer tgkplvshhp pisafhaegl nndfifhgsi ypklkfwgks
 661 veaepkgtit lellehneay twtnptccvh niivgklwie qygnveiinh ktgdkcvlnf
 721 kpcglfgkel hkvegyiqdk skkklcalyg kwteclysvd patfdaykkn dkknteekkn
 781 skqmstseel dempvpdses vfiipgsvll wriaprppns aqmynftsfa mvlnevdkdm
 841 esvipktdcr lrpdiramen geidqaseek krleekqraa rknrskseed wktrwfhqgp
 901 npyngaqdwi ysgsywdrny fnlpdiy
```

FIGURE 12G. Homo sapiens oxysterol binding protein-like protein 2 (OSBPL2), Nucleic acid sequence, transcript variant 1 (SEQ ID NO: 17)

```
   1 agtgggtcgc gggcctacgg ggcgggggcg gggcggcagt gagctcggcc ggcaaccgag
  61 ggacccgcgt ccagatcttc agtgtctatt ggatttttcc aagagaaagt ttgtaaaatt
 121 ccttacactg tagatgtgga tcagatacga tgattcagta gaagagcaca tgtcaggggc
 181 agtggaggct ggctgctgaa ggatgaacgg agaggaagaa ttctttgatg ccgtcacaga
 241 ggcaaatcag aaagtcacgg gaatgattga cttagacacc agcaaaaata ataggattgg
 301 gaaaactggg gagaggccct ctcaagagaa cggaattcag aaacacagga catcgctgcc
 361 ggctcccatg ttcagcagaa gcgacttcag cgtgtggacc atcctgaaga agtgtgttgg
 421 cctggagctg tccaagatca cgatgccaat cgccttcaac gagcctctga gcttcttgca
 481 gcggatcacg gagtacatgg agcacgtgta cctcatccac agggcctcct gccagcccca
 541 gcccctggag aggatgcagt ctgtggctgc ttttgctgtt tcggctgtgg cttcccagtg
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 601 ggagaggacc ggcaaaccat ttaatccact cttgggagaa acgtatgaat taatcaggga
 661 agatttagga ttcagattta tatcggaaca ggtcagtcac caccccccca tcagtgcgtt
 721 ccactcggaa ggtctcaacc atgacttcct gttccatggc tccatctacc ccaagctcaa
 781 gttctggggc aaaagcgtgg aggcggagcc ccgaggcacc atcaccctgg agctgctcaa
 841 acataatgaa gcctacacct ggaccaaccc cacctgctgc gtccacaacg tcatcatcgg
 901 gaagctgtgg atagagcagt atgggacagt ggagatttta aaccacagaa ctggacataa
 961 gtgtgtgctt cactttaaac cgtgtggatt atttggaaaa gaacttcaca aggtggaagg
1021 acacattcaa gacaaaaaca aaaagaagct ctttatgatc tatggcaaat ggacggaatg
1081 tttgtggggc atagatcctg tttcgtatga atccttcaag aagcaggaga ggagaggtga
1141 ccacctgaga aaggccaagc tggatgaaga ctccgggaag gctgacagcg acgtggctga
1201 cgacgtgcct gtggcccagg agaccgtgca ggtcattcct ggcagcaagc tgctctggag
1261 gatcaacacc cggcccccca actctgccca gatgtataat ttcaccagtt tcactgtgag
1321 cctcaacgag ctggagacag gcatggagaa gaccctgcca cccacggact gccgcctgcg
1381 ccctgacatc cgcggcatgg agaatggcaa catggatctg gccagccagg agaaggagcg
1441 gctggaggag aagcagagag aagcacggag ggagcgggcc aaggaggagg cagagtggca
1501 gacgaggtgg ttctacccag gcaataaccc ctacactggg acccccgact ggttgtatgc
1561 aggggattac tttgagcgga atttctccga ctgcccagat atctactgag ggcctggagg
1621 ggcctggggc ccgggaccgg aggctgacga ggctggactt cctcgagtgg ccactgtgag
1681 cctcgtcaca gcagaaacca acttttctaa cgactgagtt cgcggagata gcatcatccc
1741 tgatcaagga tgtaattcta attaactgtt gattgccaaa catttcactc tgctgtgccg
1801 tctcttcata aagcttcact tgggatcatc gtcttcatta aggtttcaac agggaaattc
1861 ttcacggcgc ccttttatgt ggcagaaatc agctggggct tgtttagctt ccagcacact
1921 ctcagtcata gcatgtgtag ctaaaggaag taatgggaag gggttcatgt tctctttata
1981 atgcagtggc aaaaggttct gaaagccttt taaactcgaa ccagtggggg aaagatggat
2041 cttgaagcta atcctgcaga gagttttata gaggccaggg attgccttct aaattatgat
2101 aaaacagaag tgaagagttt cagagcatca gattgagtga aaagttgtca gattctgtat
2161 tttttaacaa tcttcaataa tgtaaagatt acttttaaaa tatttaagtt aaaactactt
2221 gaatagtatt ttgctgaaga gcaagatatg cattaatcac cggttttata ctgtccaaaa
2281 tgaagcatcc ccgtgacaaa ccagagtggg cagaagcatc gagagcgtga caggaaatcc
2341 caagactgct tccgcctcag aggcgtcccg gctgcgattc gctgccctgt tgtcagtgag
2401 gcctggctgt caccgcacac cgcgtccgtg tctccagggg gttccctttct tctcacacgt
2461 cgcgtgtacc catagcactc ttgtgtttct gtttttccca gtatgcatgt ttaaaatagа
2521 agtgacaaga atcacatccg gttgtgtcct gtgggagggt cagaggcaga atctacttac
2581 agtggtgtaa ttaaagttat ttaaccaaaa ataggtatgt gtccatctca gcattcacct
2641 ttatcaagtg actgattttt ttttcttttc tttccttttt ttttttttt tgagacggag
2701 tttcactctt gttgcccagg ctggagtgca atggcatgat ctcggctcac cgcaacctcc
2761 gcctcccggg ttcaagcgat tctcctgcct cagcctccca agtagctggg attacaggca
2821 cgcgccacca cacctggctg attttgtatt tttagtagac acgggttttc accatgttgg
2881 tcaggctggt ctcaaactcc cgacctcaag tagtctgcct gcctcaacct cccaaagtgc
2941 tgggattaca ggcgtgagcc actgcgcctg gccgtgactg attttttttc atgtagaatt
3001 gtcaacacga gagatcacag tggagcactt tgaaagaccg tcggttgtgt gcacgcacgc
3061 acacactcat gcacacgctg acacgcggtt gcatggagtc caggttactc aggccggcac
3121 ttctgagtga caggtgccac ctgcgtgtgt cttggcgtcc acatcacacc tgtgacggaa
3181 gcacttctgg aagtgaacac tcgttttgaa agcttgattt tgtagctttg gaagctggaa
3241 gcgatggtgt ttggtgccga gtcctgtgtc atcctcgggg cctatgagct ccgtaccagc
3301 cactcaaaag tgtctgaaca gaaccgctcc gtgactggta gctgggtctg aggattcagg
3361 attgtggcgt tattcaaaga ggagactttg aaattccccg atggctggaa tgtggagccc
3421 aggtgcctct ggtggagggt catctgcttt tccagactgt ggttgtgaac cggctccttc
3481 tccaagaaag gttgcaagct gagaacatcc agaggtgaga ctcagacaca ttgaaagtga
3541 ctgcatttag ggaggtttaa cgagttctta ctgatcattc cacttgttac tggttaagat
3601 aatttgccca cgggtttgtt tccaagtcct cttctaggac caggctcctg gtatttcagg
3661 ggctggttgg ctgcacagac agcccctctt ctgctgtcct tgaggacaga caccaaacca
3721 gaggtggagg aagaacggta ggaaggctga tggcaaaagc ggctgtgtgt cgaggttatt
3781 ttaacttttt actacttttt gttactgttt ctgcaaatgc taacacataa accatgacct
3841 aacttttgtc accttggata tctattgaat gttaaacatc tctaataaag atggccacca
3901 cttaatgtgt ggaaagtgat ggccttctcg tgggc
```

FIGURE 12H. Homo sapiens oxysterol-binding protein-like protein 2 (OSBPL2), Amino acid sequence, isoform 1 (SEQ ID NO: 18)

```
  1 mngeeeffda vteanqkvtg midldtsknn rigktgerps qengiqkhrt slpapmfsrs
 61 dfsvwtilkk cvglelskit mpiafnepls flqriteyme hvylihrasc qpqplermqs
121 vaafavsava sqwertgkpf npllgetyel iredlgfrfi seqvshhppi safhseglnh
181 dflfhgsiyp klkfwgksve aeprgtitle llkhneaytw tnptccvhnv iigklwieqy
241 gtveilnhrt ghkcvlhfkp cglfgkelhk veghiqdknk kklfmiygkw teclwgidpv
301 syesfkkqer rgdhlrkakl dedsgkadsd vaddvpvaqe tvqvipgskl lwrintrppn
361 saqmynftsf tvslneletg mektlpptdc rlrpdirgme ngnmdlasqe kerleekqre
421 arrerakeea ewqtrwfypg nnpytgtpdw lyagdyfern fsdcpdiy
```

FIGURE 12I. Homo sapiens oxysterol binding protein-like protein 2 (OSBPL2), Nucleic acid sequence, transcript variant 2 (SEQ ID NO: 19)

```
   1 agtgggtcgc gggcctacgg ggcgggggcg gggcggcagt gagctcggcc ggcaaccgag
  61 ggacccgcgt ccagatcttc agtgtctatt ggatttttcc aagagaaagt ttgtaaaatt
 121 ccttacactg tagatgtgga tcagatacga tgattcagta gaagagcaca tgtcaggggc
 181 agtggaggct ggctgctgaa ggatgaacgg agaggaagaa ttctttgatg ccgtcacagg
 241 ctttgattct gataactctt ctggggaatt ttcagaggca aatcagaaag tcacgggaat
 301 gattgactta gacaccagca aaaataatag gattgggaaa actggggaga ggccctctca
 361 agagaacgga attcagaaac acaggacatc gctgccggct cccatgttca gcagaagcga
 421 cttcagcgtg tggaccatcc tgaagaagtg tgttggcctg gagctgtcca agatcacgat
 481 gccaatcgcc ttcaacgagc ctctgagctt cttgcagcgg atcacggagt acatggagca
 541 cgtgtacctc atccacaggg cctcctgcca gccccagccc ctggagagga tgcagtctgt
 601 ggctgctttt gctgtttcgg ctgtggcttc ccagtgggag aggaccggca aaccatttaa
 661 tccactcttg ggagaaacgt atgaattaat cagggaagat ttaggattca gatttatatc
 721 ggaacaggtc agtcaccacc cccccatcag tgcgttccac tcggaaggtc tcaaccatga
 781 cttcctgttc catggctcca tctaccccaa gctcaagttc tggggcaaaa gcgtggaggc
 841 ggagccccga ggcaccatca ccctggagct gctcaaacat aatgaagcct acacctggac
 901 caaccccacc tgctgcgtcc acaacgtcat catcgggaag ctgtggatag agcagtatgg
 961 gacagtggag attttaaacc acagaactgg acataagtgt gtgcttcact ttaaaccgtg
1021 tggattattt ggaaaagaac ttcacaaggt ggaaggacac attcaagaca aaaacaaaaa
1081 gaagctcttt atgatctatg gcaaatggac ggaatgtttg tggggcatag atcctgtttc
1141 gtatgaatcc ttcaagaagc aggagaggag aggtgaccac ctgagaaagg ccaagctgga
1201 tgaagactcc gggaaggctg acagcgacgt ggctgacgac gtgcctgtgg cccaggagac
1261 cgtgcaggtc attcctggca gcaagctgct ctggaggatc aacacccggc cccccaactc
1321 tgcccagatg tataatttca ccagtttcac tgtgagcctc aacgagctgg agacaggcat
1381 ggagaagacc ctgccaccca cggactgccg cctgcgccct gacatccgcg gcatggagaa
1441 tggcaacatg gatctggcca gccaggagaa ggagcggctg gaggagaagc agagagaagc
1501 acggagggag cgggccaagg aggaggcaga gtggcagacg aggtggttct acccaggcaa
1561 taacccctac actgggaccc ccgactggtt gtatgcaggg gattactttg agcggaattt
1621 ctccgactgc ccagatatct actgagggcc tggaggggcc tggggcccgg gaccggaggc
1681 tgacgaggct ggacttcctc gagtggccac tgtgagcctc gtcacagcag aaaccaactt
1741 ttctaacgac tgagttcgcg gagatagcat catccctgat caaggatgta attctaatta
1801 actgttgatt gccaaacatt tcactctgct gtgccgtctc ttcataaagc ttcacttggg
1861 atcatcgtct tcattaaggt ttcaacaggg aaattcttca cggcgccctt ttatgtggca
1921 gaaatcagct ggggcttgtt tagcttccag cacactctca gtcatagcat gtgtagctaa
1981 aggaagtaat gggaaggggt tcatgttctc tttataatgc agtggcaaaa ggttctgaaa
2041 gccttttaaa ctcgaaccag tgggggaaag atggatcttg aagctaatcc tgcagagagt
2101 tttatagagg ccagggattg ccttctaaat tatgataaaa cagaagtgaa gagtttcaga
2161 gcatcagatt gagtgaaaag ttgtcagatt ctgtattttt taacaatctt caataatgta
2221 aagattactt ttaaaatatt taagttaaaa ctacttgaat agtattttgc tgaagagcaa
2281 gatatgcatt aatcaccggt tttatactgt ccaaaatgaa gcatccccgt gacaaaccag
2341 agtgggcaga agcatcgaga gcgtgacagg aaatcccaag actgcttccg cctcagaggc
```

```
2401 gtcccggctg cgattcgctg ccctgttgtc agtgaggcct ggctgtcacc gcacaccgcg
2461 tccgtgtctc caggggttc ctttcttctc acacgtcgcg tgtacccata gcactcttgt
2521 gtttctgttt ttcccagtat gcatgtttaa aatagaagtg acaagaatca catccggttg
2581 tgtcctgtgg gagggtcaga ggcagaatct acttacagtg gtgtaattaa agttatttaa
2641 ccaaaaatag gtatgtgtcc atctcagcat tcacctttat caagtgactg attttttttt
2701 cttttctttc ctttttttt ttttttgag acggagtttc actcttgttg cccaggctgg
2761 agtgcaatgg catgatctcg gctcaccgca acctccgcct cccgggttca agcgattctc
2821 ctgcctcagc ctcccaagta gctgggatta caggcacgcg ccaccacacc tggctgattt
2881 tgtatttta gtagacacgg gttttcacca tgttggtcag gctggtctca aactcccgac
2941 ctcaagtagt ctgcctgcct caacctccca aagtgctggg attacaggcg tgagccactg
3001 cgcctggccg tgactgattt tttttcatgt agaattgtca acacgagaga tcacagtgga
3061 gcactttgaa agaccgtcgg ttgtgtgcac gcacgcacac actcatgcac acgctgacac
3121 gcggttgcat ggagtccagg ttactcaggc cggcacttct gagtgacagg tgccacctgc
3181 gtgtgtcttg gcgtccacat cacacctgtg acggaagcac ttctggaagt gaacactcgt
3241 tttgaaagct tgattttgta gctttggaag ctggaagcga tggtgtttgg tgccgagtcc
3301 tgtgtcatcc tcggggccta tgagctccgt accagccact caaaagtgtc tgaacagaac
3361 cgctccgtga ctggtagctg ggtctgagga ttcaggattg tggcgttatt caaagaggag
3421 actttgaaat tccccgatgg ctggaatgtg gagcccaggt gcctctggtg gagggtcatc
3481 tgcttttcca gactgtggtt gtgaaccggc tccttctcca agaaaggttg caagctgaga
3541 acatccagag gtgagactca gacacattga aagtgactgc atttagggag gtttaacgag
3601 ttcttactga tcattccact tgttactggt taagataatt tgcccacggg tttgtttcca
3661 agtcctcttc taggaccagg ctcctggtat ttcaggggct ggttggctgc acagacagcc
3721 cctcttctgc tgtccttgag gacagacacc aaaccagagg tggaggaaga acggtaggaa
3781 ggctgatggc aaaagcggct gtgtgtcgag gttattttaa ctttttacta ctttttgtta
3841 ctgtttctgc aaatgctaac acataaacca tgacctaact tttgtcacct tggatatcta
3901 ttgaatgtta aacatctcta ataaagatgg ccaccactta atgtgtggaa agtgatggcc
3961 ttctcgtggg c
```

FIGURE 12J. Homo sapiens oxysterol binding protein-like protein 2 (OSBPL2), Amino acid sequence, isoform 2 (SEQ ID NO: 20)

```
  1 mngeeeffda vtgfdsdnss gefseanqkv tgmidldtsk nnrigktger psqengiqkh
 61 rtslpapmfs rsdfsvwtil kkcvglelsk itmpiafnep lsflqritey mehvylihra
121 scqpqplerm qsvaafavsa vasqwertgk pfnpllgety eliredlgfr fiseqvshhp
181 pisafhsegl nhdflfhgsi ypklkfwgks veaeprgtit lellkhneay twtnptccvh
241 nviigklwie qygtveilnh rtghkcvlhf kpcglfgkel hkveghiqdk nkkklfmiyg
301 kwteclwgid pvsyesfkkq errgdhlrka kldedsgkad sdvaddvpva qetvqvipgs
361 kllwrintrp pnsaqmynft sftvslnele tgmektlppt dcrlrpdirg mengnmdlas
421 qekerleekq rearrerake eaewqtrwfy pgnnpytgtp dwlyagdyfe rnfsdcpdiy
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